The neurodevelopmental outcomes of perinatally HIV-infected children on different antiretroviral treatment (ART) strategies

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Declaration:

I declare that the entirety of the work contained herein is my own original work, except when otherwise stated, that I am the authorship owner thereof and that I have not previously submitted it, in its entirety or in part, for the purpose of obtaining any qualification.

I acknowledge Professor Martin Kidd who performed the statistical analyses for this study and professor Steve Innes as a first co-author of one paper.

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Summary:

At the commencement of this study, it was apparent that antiretroviral therapy (ART) improved neurodevelopmental outcomes of children infected with HIV. Little was known about the long-term outcomes in infants who commenced early ART, or whether there would be consequences of temporary ART interruption.

We conducted a prospective, longitudinal, observational study to determine the neurodevelopmental outcomes of children perinatally infected with HIV on different ART strategies from the Children with HIV Early antiRetroviral treatment (CHER) trial. We compared the outcomes of children whose ART was deferred to children who started early ART but with planned interruption of treatment.

We also assessed the neurodevelopmental outcomes at 11months of age in a cohort of children perinatally infected with HIV, who started ART within the first few weeks of life.

The Griffiths mental development scales (GMDS) were used to assess neurodevelopment at 11, 20, 30, 42 and 60 months, and the Beery-Buktenica developmental tests of visual motor integration were performed at 60 months.

HIV-exposed uninfected (HEU) and HIV-unexposed (HU) children from similar neighbourhoods were enrolled for comparison. Mixed model repeated measures were used to compare groups over time.

We found that children whose ART was deferred, had worse locomotor and general development in the first year of life compared to those who started treatment early and whilst asymptomatic with planned interruption. However, by five years of age the GMDS scores were similar.

Children who started very early ART at a median age of 6 days, had similar GMDS scores at 11 months of age to the early treatment arm on CHER, who had started ART at median of 8 weeks.

During the study we noted that children developed HIV encephalopathy, despite being on ART, including some with viral suppression. These children were followed for a median

or 6.2 years and most recovered. This suggested a temporary insult, possibly due to inflammation associated with immune reconstitution that then resolved over time.

An important finding was the visual perceptual deficit noted in HIV-infected children, compared to uninfected controls at 5 years of age.

This study demonstrated that initiation of ART at a young age in an asymptomatic HIV-infected cohort had encouraging neurodevelopmental outcome at 5 years, apart from visual perception which was noted regardless of ART treatment strategy. Planned treatment interruption did not affect neurodevelopmental outcome by 5 years of age, but this was with careful clinical surveillance.

Longer-term outcomes in older children would continue to provide further knowledge on ART treatment strategies.

Opsomming:

Met die aanvang van hierdie studie, was dit duidelik dat antiretrovirale terapie (ART) die neurologiese ontwikkelings uitkomste van kinders met HIV besmetting verbeter het. Daar was 'n gebrek aan inligting oor die langtermyn uitkomste van kinders wat vroeë ART behandeling gekry het, en of daar gevolge sou wees vir tydelike onderbreking van ART.

Ons het 'n voornemende, langtermyn, observasionele studie na die neurologiese ontwikkelings uitkomste van kinders wat perinataal met MIV besmet was, en op verskillende ART behandelings strategieë, gedoen om die uitslae te vergelyk met die van kinders met MIV en wat op vroeë antiretrovirale behandeling is. Ons het die uitkomste van kinders wie se ART behandeling uitgestel is vergelyk met die van kinders wat ART behandeling vroeg begin het en beplande onderbreking van behandeling ondergaan het, in die Children with HIV early antiretroviral treatment (CHER) studie. Ons het ook die neurologiese ontwikkeling uitkomste op 11 maande van ouderdom in 'n groep van kinders wat perinataal met MIV besmet was, en wat ART binne die eerste paar weke van die lewe begin het, geassesseer.

Die Griffiths Mental Development Scales (GMDS) is gebruik om die neurologiese ontwikkeling van kinders op 11, 20, 30, 42 en 60 maande te bepaal, en die Beery-Buktenica ontwikkelings toetse van visuele motoriese integrasie is uitgevoer op 60 maande. MIV-blootgestelde onbesmette (HEU) en MIV-onbesmette (HU) kinders van soortgelyke buurte is ingeskryf vir 'n vergelyking. 'n Gemengde model herhaal maatreëls is gebruik om groepe oor ‘n tydperk te vergelyk.

Ons het gevind dat kinders wie se ART uitgestel is, slegter lokomotoriese en algemene ontwikkeling getoon het in die eerste jaar, in vergelyking met diegene wat behandeling vroeg begin het wanneer hulle asimptomaties was en wat beplande onderbreking van behandeling ondergaan het. Tog op vyf jaar van ouderdom was die GMDS tellings soortgelyk.

Kinders wat vroeë ART behandeling by 'n gemiddelde ouderdom van 6 dae begin, het soortgelyke GMDS tellings op 11 maande van ouderdom gehad as die van die vroeë behandeling arm op CHER, wat ART by mediaan van 8 weke begin het.

Tydens die studie is opgemerk dat kinders MIV enkefalopatie ontwikkel, ten spyte daarvan dat hulle op ART behandeling was, insluitend 'n paar wat virale onderdrukking gehad het. Hierdie kinders is opgevolg vir 'n mediaan van 6,2 jaar en die meeste van hulle het herstel. Hierdie was 'n aanduiding van tydelike skade, waarskynlik weens inflammasie, gepaartgaande met immuun-herstel, wat daarna met verloop van tyd opgeklaar het

'n Belangrike bevinding was die opmerking van 'n visuele perseptuele gebrek in kinders wat met MIV besmet is, in vergelyking met die onbesmette kontroles op 5 jaar van ouderdom.

Hierdie studie het getoon dat die aanvang van ART behandeling op 'n jong ouderdom, in 'n asimptomatiese MIV-besmette groep, bemoedigende neurologiese uitkomste op 5 jaar getoon het. Die bevinding is tenspyte van visuele persepsie wat ongeag die ART behandelings strategie opgemerk was. Beplande behandeling onderbreking het geen invloed op die neurologiese uitkomste teen die ouderdom van 5 jaar aangedui nie, maar hierdie met noukeurige kliniese toesig.

Die langer termyn uitkomste in ouer kinders sal voortaan verdere kennis verskaf oor ART behandelings strategieë.

Dedication:

I dedicate this work to the children of Africa.

May the sun always shine on you.

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Introduction

Neurological manifestations during the early years of the HIV epidemic

Human immunodeficiency virus (HIV) infection in children was first described in 1983, antedating effective treatment by 12 years.[1] Early clinical descriptions of HIV infection included neurological manifestations as part of the disease process. Epstein was the first to recognize the HIV's impact on the developing brain. He described developmental delays, predominantly in motor milestones, perceptual motor abilities and expressive speech.[2]. CT scans of HIV infected children's brains often showed cerebral atrophy with symmetrical calcification of the basal ganglia and/or periventricular white matter. These findings were mostly associated with advanced symptomatic disease.[2]

Delay of motor milestones is a prominent feature in younger HIV infected children.[3] Neurodevelopmental delay can be as a direct result of the effects of HIV in the brain, or from indirect effects such as systemic illness, poor nutrition, maternal illnesses and psychosocial stressors. Secondary central nervous system (CNS) complications may also occur e.g. CNS infections, neoplasms or cerebrovascular accidents.[4-6] Neurological signs, especially if progressive, predicted mortality in HIV infected children.[2, 7]

HIV encephalopathy

HIV penetrates the infant's brain very early, at time when the brain is growing rapidly and vulnerable to insults.[8-10] The brain is in its most rapid growth period during the first 20 postnatal weeks and by the age of 3 years, the average weight is almost that of an adult. Branching, myelination and organisation of neurons occur, along with selective pruning and apoptotic programming.[11]

HIV entry into the CNS is through infected monocytes and causes neuronal injury directly, or indirectly through the host inflammatory response, which may affect active synaptogenesis and organization of neurones in the developing brain.[12] As a result, neurological insults occur with variable clinical manifestations and the age for developing HIV encephalopathy (HIVE) is variable.[2, 5, 9, 13, 14] Younger children are particularly vulnerable with those under 3 years of age demonstrating higher rates failure to thrive, development and cognitive abilities.[15]

Due to varying descriptions and definitions by early investigators, a working group of the American academy of Neurology AIDS task force in 1991 developed appropriate nomenclature and described *HIV-1 associated progressive encephalopathy of childhood* with criteria for clinical diagnosis.[16] (See appendix 1)

Three additional HIVE patterns were also described: Static (no further loss of developmental milestones but following a trajectory slower than normal development), plateau (no new developmental skills acquired) and subacute progressive (loss of previously acquired developmental skills).[5, 10]

Clinical signs for HIVE occurring in first year of life, presumed due CNS insults from in-utero HIV infection and CNS insults were also described.[14] These include:

- encephalopathy that developed before 1 year of age
- reduction of intrauterine brain growth
- very low levels of cerebrospinal fluid (CSF) HIV-1 RNA
- occurrence at a higher than expected CD4+ T-cell percentages and counts
- not prevented by intrauterine exposure to Zidovudine

Effects of antiretroviral therapy:

Antiretroviral therapy demonstrated benefits for neurodevelopment and cognitive function in children with HIVE, first AZT and then combined ART.[17-20] However not all deficits were reversible, and children would revert to a static encephalopathy, those with an early AIDS-defining (Centers for Disease Control and Prevention (CDC) class C) illness.[21-24]

Faye et al showed that early ART multidrug therapy (before the age of 6 months) had an improved effect on neurodevelopment compared to when ART was deferred until after 6 months of age.[25] There were few reports on the neurodevelopmental outcome of children in in Africa. Van Rie et al described the neurodevelopmental benefit of children enrolling in care and starting ART at a younger age.[26]

Starting ART as early as possible after the primary infection would prevent the early effects of the virus on the immune system and neurodevelopmental deficits.

The CHER Trial:

Although there was evidence for improved outcomes after starting ART early, concern remained about the risk for resistance after exposure to drugs used for prevention of mother to child transmission (PMTCT) and the potential for toxicity or resistance associated with lifelong ART. The Children with HIV Early antiRetroviral treatment (CHER) trial was designed to compare early time-limited ART with deferred ART. The strategy was that early time limited ART initiated close to primary infection would prevent disease progression and safely allow a subsequent period off ART, thus preserving future treatment options and preventing toxicity.[27, 28]

HIV+ infants were recruited between 6 and 12 weeks of age from community clinics in 2005-2007, from the prevention of mother to child treatment (PMTCT) program, at two South African study sites: The Children's Infectious Diseases Clinical Research Unit at Tygerberg Academic Hospital, Cape Town and the Perinatal HIV Research Unit in Soweto, Johannesburg. Those with CD4 ≥25% were randomized to one of three treatment strategies:

i) ART deferred until indicated (ART-Def),
ii) Early limited ART for 40 weeks (ART-40W),
iii) Early limited ART for 96 weeks (ART-96W).

Continuous ART was initiated (in ART-Def) and reinitiated (in ART-40W and ART-96W) when the CD4 declined <25% in the first year of life and <20% thereafter, or for severe stage B or C disease according to CDC standards. After a median of 4.8 years, the superiority of early time-limited over deferred continuous ART was confirmed.[27, 28] The CHER trial started in July 2005 and formally ended on 31 August 2011. Of the 377 infants reported in the main trial, 115 were enrolled in Cape Town.

A nested neurodevelopmental sub-study was conducted in Cape Town. Outcomes from this neurodevelopmental sub-study are the main focus of this PhD, results are reported in chapters 2, 3 and 4.

Neurodevelopmental assessment in young South African children.

The Griffiths Mental Development Scales (GMDS) was selected as the assessment tool of choice. [29] There were no comprehensive neurodevelopmental assessment tools standardised for South African children. The advantage of the GMDS is that it assesses children from 0-8 years, has been used extensively in South African children and translated into the local languages: Xhosa and Afrikaans. The most recent edition (GMDS-ER) was updated involving researchers from Nelson Mandela Metropolitan University, South Africa and most test items were appropriate for South African Children.[30, 31] However, standard scores and age equivalents were developed from appropriately developing British children.[30, 32]

The Beery-Buktenica developmental tests of visual-motor integration, visual perception and motor-coordination (6th edition) were also administered.[33] These tests are commonly used in South Africa in clinical and research settings.

Since the test results have foreign normative values, HIV uninfected control groups were enrolled for comparison. Children perinatally unexposed to HIV and children perinatally exposed to HIV but uninfected from the same communities as the CHER children, were enrolled from an interlinked vaccine study. [34] As children enrolled on this study were from impoverished environments, we expected an increasing gap between acquired abilities over time. Therefore, neurodevelopmental trajectories of children from similar neighbourhoods were essential for correctly identifying neurodevelopmental delay. Illustrating the importance of controls, we described a decline in their scores.[35] Without this reference group a decline in scores would have otherwise been ascribed to HIV.

Central theme of this thesis:

Is early treatment better for early childhood neurodevelopment?

Study hypothesis: The neurodevelopmental outcome of children starting ART early will be better than that of children where the treatment has been delayed and will be similar to a control (HIV uninfected) group.

This work is divided into five chapters to answer this question.

Chapter 1:

A review of the effects of HIV on neurodevelopment

An extensive literature review was conducted to summarise the neurodevelopmental outcomes of children infected with HIV including those on a variety of ART regimens and from a wide range of resource availability settings.

A number of deficits were identified in children infected with HIV despite ART. This review highlighted the neurocognitive disease burden for future generations of school-age children and adolescents.

Chapter 2:

The effect of early antiretroviral therapy on neurodevelopment

The questions from 2005 were: Is it safe to start ART early in asymptomatic children? What about toxicity or developing resistance? The prevailing opinion in South Africa at the time, particularly amongst politicians and the media, was that ART was toxic and HIV diagnosis was highly stigmatised.[36, 37] These considerations led to the international community announcing the Durban Declaration in 2000.[38]

In chapter 2 the early neurodevelopmental outcomes at 11 months of perinatally HIV infected children on the CHER trial, who started ART early were compared to those whose treatment was deferred. Participants were randomised into early and deferred treatment

groups according to the CHER protocol, which strengthened the study design. Of the early ART group 59/64 (92%) were assessed before the planned interruption phase.

Hypothesis: Early ART would be better for neurodevelopmental outcomes in children with HIV.

Chapter 3:

The effect of planned treatment interruption on neurodevelopment

In this prospective, longitudinal, observational study of the neurodevelopmental outcomes of children on the different treatment arms of the CHER trial were compared.

The Griffiths mental development scales (GMDS) were performed at 11, 20, 30, 42 and 60 months, six subscales and a global score, and the Beery-Buktenica developmental tests for visual motor integration at 60 months.

Primary Objective: To compare the neurodevelopmental outcome of 5 groups of children over 5 years:

1. ART is deferred until clinical or immunological disease progression (effect of ART on the CNS is delayed, but effect of the virus is observed).
2. ART is started early but is given for a shorter period (until 1st birthday)
3. ART is started early and is given for a longer period (until 2nd birthday)
4. Controls who are perinatally HIV exposed and uninfected
5. Controls who are perinatally HIV unexposed and uninfected

Hypothesis: There will not be a negative effect on neurodevelopmental outcomes of perinatally HIV infected children interrupting ART after an early limited period on ART. Neurodevelopment will be similar to uninfected controls.

Appendix 2 provides a description of the sample size calculation.

Chapter 4:

Clinical trajectory of HIV encephalopathy

The CHER trial was the first large randomized study of children perinatally infected with HIV who commenced early ART. We noted that the trajectory of clinical signs for HIVE was different to cases described in the pre-ART era. Children on early ART developed HIVE, although with milder deficits and subsequent recovery. This had not previously been described in detail. This paper was co- first authored with Dr Steve Innes.

Chapter 5:

Effects of starting very early antiretroviral therapy on neurodevelopment

Hypothesis: The early neurodevelopment of infants starting ART in the first few days of life will be within the normal range.

Contemporary thinking was that immediate ART could prevent or decrease the size of the HIV reservoir in brain.[39] However there were concerns of toxicity to the rapidly developing neonatal brain, especially if ART over-dosing occurred due to difficulty measuring syrups. [40] In chapter 5, the neurodevelopmental outcome at 11.5 months of a cohort of infants, who started ART at a median age of 6 days of age is described.

Chapter 1

Review of literature on the neurodevelopmental outcomes of children perinatally infected with HIV

Unlike many other chronic illnesses, there is no classical phenotype of neurodevelopmental deficits in children perinatally infected with HIV.

Neurodevelopmental outcome studies are not uniform, and outcomes are varied. This may be due to multiple factors, including timing of infection (in utero, intra-partum or post-natal) and starting treatment, different ART regimens, viral properties, host conditions, assessment tools and other environmental factors.

A comprehensive literature survey was conducted to summarise evidence on neurocognitive deficits due to HIV infection and was published in a supplement on adolescent HIV in the Journal of the International AIDS Society. The outcomes of school-aged children were described as there was very little published data on adolescent outcomes at that time (2013).

The domains most likely to be affected in children living with HIV infection were:

- General cognition
- Processing speed
- Visual-spatial abilities

We noted that neurocognitive deficits may be subtle and not as obvious as HIV encephalopathy described in the pre-ART era; CNS insults from HIV were variable as there were multiple causal factors; those with AIDS defining illnesses had worse outcomes; and children perinatally infected with HIV were at higher risk for psychiatric and mental health problems.

This review described the substantial and underappreciated burden of HIV on the neurodevelopmental outcomes of children and encouraged all physicians to be on the alert for neurodevelopmental deficits.

This publication has been cited 137 times (PubMed September 2019), probably because it is one of the first to summarise information that was not readily available.

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JIAS Journal of the International AIDS Society

Review article

Neurodevelopment in perinatally HIV-infected children: a concern for adolescence

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Abstract

Globally, an estimated 3.4 million children are living with HIV, yet little is known about the effects of HIV and antiretroviral treatment (ART) on the developing brain, and the neurodevelopmental and behavioural outcomes of perinatally HIV-infected (PHIV+) adolescents.

We reviewed the literature on neurodevelopmental outcomes in PHIV+ children and adolescents, and summarized the current evidence on behaviour, general cognition, specific domains, hearing and language, school performance and physical disabilities due to neurological problems.

Evidence suggests that PHIV+ children do not perform as well as controls on general cognitive tests, processing speed and visual–spatial tasks, and are at much higher risk for psychiatric and mental health problems. Children with AIDS-defining diagnoses are particularly at risk for poorer outcomes.

A striking finding is the lack of published data specific to the adolescent age group (10–25 years), particularly from resource-constrained countries, which have the highest HIV prevalence. In addition, extreme heterogeneity in terms of timing and source of infection, and antiretroviral experience limits our ability to summarize findings of studies and generalize results to other settings.

Due to the complex nature of the developing adolescent brain, environmental influences and variation in access to ART, there is an urgent need for research on the longitudinal trajectory of neurodevelopment among children and adolescents perinatally infected with HIV, especially in high burden resource-constrained settings.

Keywords: adolescents; children; perinatally HIV infected; neurodevelopment; neurocognitive; neurological; hearing; executive function.

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Introduction

An estimated 3.4 million children are living with HIV worldwide [1], 28% of whom have started antiretroviral therapy (ART) [2]. Yet, little is known about the effects of HIV and ART on the developing brain and the neurodevelopmental outcomes of perinatally HIV-infected (PHIV+) adolescents.

In neuropsychological terms, adolescence spans the age range of 10–25 years [3], which in 2013 includes those born between 1988 and 2003. Over this time period, the management of PHIV+ infants, children and adolescents changed dramatically. Before the introduction of ART in 1995, 50% of PHIV+ children died before the age of two [4], with a few slow progressors surviving to adolescence [5]. Prior to 1997, children in Europe and the United States may have received multiple antiretroviral regimens, including those that would now be considered suboptimal therapy. In 1997, combination ART was introduced in the United States. Since 2004, access to ART has expanded rapidly in resource-poor settings and depending on the country, 28–80% of treatment-eligible children have initiated ART [2].

Context-specific differences in access to ART over the past two decades have resulted in great variability in disease severity and in exposure to ART among PHIV+ adolescents: some started ART soon after HIV infection, prior to clinical diagnosis of neurodevelopmental delay [6]; some initiated ART after the diagnosis of HIV encephalopathy [7,8], resulting in neurological deficits that remained permanent despite ART [9,10]; other PHIV+ adolescents are slow progressors and remain ART-naïve as they have not yet reached the ART eligibility threshold [2]. The source and time of infection cannot always be determined in HIV+ adolescents, especially in settings with generalized HIV epidemics.

There is also substantial heterogeneity in the literature in terms of the age of study participants. Most published studies have focused on younger children aged 6–12 years [11–13] or 7–16 years [14], crossing from childhood to adolescence. The issue is further compounded by the measures used to assess functionally relevant outcomes for PHIV+ adolescents in diverse cultural settings. In some settings, the emphasis may be on achieving good school grades to

maximize employment options, while in other settings adolescents may be more concerned about starting a family and providing resources to support a household or extended family.

Given this extreme heterogeneity in the age of study participants, severity of disease, antiretroviral experience, and the definition and measurement of outcomes, we reviewed the literature on neurodevelopmental outcomes in PHIV+ adolescents.

We conducted a literature search using the following key words: neurodevelopment/al, development, neurocognitive, cognitive, adolescents, youth, perinatal/vertical HIV-infected, HIV exposed, school performance, adaptive functioning, hearing and neuroimaging. We reviewed bibliographies and relevant articles from different contexts globally, limited to the most recent papers. We included all ages spanning adolescence. The original aim was to review evidence on neurodevelopmental outcomes among perinatally HIV-infected adolescents. However, the paucity of strictly adolescent data was striking. In the absence of these data, we drew on published studies of neurodevelopmental in younger children with HIV as a guide to what could be expected to impair neurodevelopment in adolescence.

Neurodevelopmental changes during adolescence

The key developmental tasks during adolescence are to develop an identity, to become more independent, and to consider the future in terms of career, relationships, families, housing, etc. [15]. Traditionally, adolescence is viewed as the age when abstract thought develops, together with improvements in memory, language, processing speed, attention and concentration [16]. A more contemporary view is that the major dimensions of cognitive development during adolescence are the refinement of executive control and the attainment of a more conscious, self-directed and self-regulating mind [17–19]. Central to these are executive function (EF) processes such as voluntary response inhibition, working memory, response planning, improved processing speed, cognitive flexibility, and rule-guided behaviour [18,19].

While the adolescent's brain does not increase substantially in volume, changes in maturation reflect reorganization of regulatory systems and correlate with neurocognitive and behavioural outcomes [17]. During adolescence, white matter increases in a linear fashion with increased myelination and re-organization with synaptogenesis and pruning, especially in the frontal lobes and prefrontal cortex, which serve as the governor of cognition and action [17,18]. Maturational changes are influenced by numerous factors including genetic and environmental factors as well as overall health status, resulting in variation between children and within the same child for the various domains of neurodevelopment. The impulsive and risk-taking behaviour of adolescents is also thought to be a consequence of the interaction of social context and the development of judgment, decision-making and internal control [17,20].

Neuropathology caused by HIV is most evident in basal ganglia and cerebral white matter. Neuronal loss is prominent in the prefrontal cortical regions, which may cause difficulty in complex mental processing [21]. These are the regions where myelination and remodelling of synaptic connections are still occurring during adolescence [22,23]. When coupled with the high risk for psychiatric difficulties in PHIV+ adolescents, the relationship between impaired EF and risk-taking behaviour can be compounded.

General cognition

The most common measure of neurodevelopmental outcome is general cognition. General cognitive assessments provide a global score of performances in various domains. In this case, appropriate perinatally HIV-unexposed (PHU) children can be used as controls [12]. Neighbourhood-matched perinatally HIV-exposed uninfected (PHEU) children can be used to control for confounding effects of prenatal HIV exposure, ART exposure and maternal illness, etc. [11,24], but they should not be seen as an ideal control group.

Table 1 summarizes recent studies of general cognition in PHIV+ children. Most neurocognitive assessment studies of PHIV+ children have been performed in the United States and Europe [14,25–27], though some studies from other continents have been published [11–13,24]. There are many differences between the study populations, with each group having particular areas of vulnerability of the brain and life experiences (e.g., higher drug abuse and lower adherence in some parts of the United States; higher poverty and lower access to comprehensive treatment in some limited-resource countries; different treatment when infants). Overall, PHIV+ children and adolescents perform more poorly in neurodevelopmental assessments than PHU controls or national norms [11,13,24], although in some studies there were no significant differences between groups [12,26,27]. For children in the United States, better cognitive outcomes have been associated with having a biological parent as caregiver, higher family income level, and higher caregiver cognitive function [14]. In PHIV+ children with less severe disease progression (WHO clinical stage I or II) and those on ART without a history of an AIDS defining illness, overall cognitive development has been found to be similar to that of PHEU children [14] although still significantly poorer than PHU children [13].

Martin and colleagues evaluated predictors of cognitive decline in older children in the United States who had been on ART for at least a year. Overall, PHIV+ children on ART remain at risk for developing CNS disease, with children with minimal to moderate CT brain scan abnormalities scoring significantly lower than children with normal scans on composite measures of cognitive ability [21]. The risk in asymptomatic adolescents was confirmed in a small pilot study which found a higher rate of neurocognitive impairment in asymptomatic adolescents compared to adults >60 years old (67 vs. 19%) [28].

Few studies have addressed the effect of ART initiation on cognitive development in PHIV+ school-age children and adolescents in low- and middle-income countries. In a cohort of Thai children, cognitive function did not improve in response to ART, even in children who achieved virological suppression and immunological recovery [11]. There were

Table 1. Summary of recent studies on general cognition in HIV-infected children

Study	Participants	Age (range)	Measure	Findings	Antiretroviral therapy
Koekkoek *et al*. 2008 [26] The Netherlands	22 PHIV+	Median 9.46 years (6–13.5)	SON-R	No gross cognitive deficits compared to normative values	Median age HAART initiation 5.6 years
Smith *et al*. 2012 USA+Puerto Rico [14]	270 PHIV+/noC 88 PHIV+/C 200 PHEU	7–16 years	WISC-IV	Scores significantly lower for PHIV+/C group after adjusting for covariates 77.8 vs. 83.4 and 83.3	Median age: first ART 0.6 years; first dual therapy 1.25 years
Blanchette *et al.* 2002 [27] Canada	14 PHIV+ 11 control siblings	6.3–14 years	WISC-R or WISC-III	Mean FISQ 91.7 vs. 100.5	12 were on ART
Ruel *et al.* 2011 Uganda [13]	93 PHIV+ 106 HIV−	Median 8.7 years (6–12)	KABC-2	PHIV+ performed worse than HIV- children	All children above WHO threshold for ART initiation
Bagenda 2006 Uganda [12]	28 HIV+ 42 HEU 37 HIV−	6–12 years	KABC	No significant difference	ART-naïve
Wood 2009 USA [25]	81 PHIV+ 38 PHIV+/C 43 PHIV+/noC	Median 15.2 years (11–24)	WISC-IV or WASI	Median FISQ of PHIV+/noC fell within normal range; Median FISQ of HIV+/C in below average range	Median age: ART initiation: 3.1 years; Median age: HAART initiation: 6.5 years
Puthanakit *et al.* 2010 [11] Thailand	39 HIV + 40 − affected 42 healthy controls	Median 9.3 years (6–12)	WISC-III	Mean FISQ of HIV+ and affected groups significantly lower than healthy controls 79 vs. 88 vs. 96 $p < 0.01$	87% on ART for median of 35 weeks (IQR 29–53)
Puthanakit *et al.* 2013 [29] Thailand, Cambodia	284 PHIV+, 155 PHEU, 164 PHU	Median age 9 years (1–12)	WISC-Thai	No difference between early and deferred ART initiation RCT arms. PHIV+ children performance worse than PHEU and PHU on IQ	Early versus deferred HAART at enrolment from 1 to 12 years of age
Hoare *et al.* 2012 South Africa [24]	12 PHIV+ 12 HIV − community controls	8–12 years	WASI: verbal performance	Mean scores: 87.8 vs. 101.2 73.7 vs. 85.7	ART-naïve

PHIV+/C Perinatally HIV-infected with a previous class C event.
PHIV+/noC Perinatally HIV-infected with no past history of class C event.
KABC Kaufman Assessment Battery for children.
KABC-2 Kaufman Assessment Battery for children, 2nd edition.
SON-R Snijders-Oomen nonverbal intelligence test for children and adolescents (abridged).
WASI Wechsler Abbreviated Scale of Intelligence.
WISC-R Wechsler Intelligence Scale for Children −Revised.
WISC-III & IV Wechsler Intelligence Scale for Children versions 3, 4. WISC-Thai Wechsler Intelligence Scale for Children Thai version.

also similar neurodevelopmental and neuropsychological outcomes in Thai and Cambodian children between early and deferred ART groups, although both groups performed worse than PHEU children [29]. A small study of younger South African children (median age five years) also failed to observe neurodevelopmental improvement following ART initiation [9].

Specific domains of cognitive development

Global cognitive scores may overlook subtle deficits in one or more areas specific to PHIV+ children and may affect their performance on a different level [26,30,31]. For example, even in PHIV+ children with global cognitive scores in the normal range, EF may be impaired, especially in children with cortical atrophy, lower fractional anisotropy of the corpus callosum and those with CD4+ counts below 500 cells/mm^3 [21,24].

Specific domains may be measured as subtests on cognitive assessments or by a test specifically designed for that purpose. The development of EF starts in childhood, but is highly important in the development of adolescents. EF is a composite of different domains including processing speed, response inhibition, working memory, response planning, cognitive flexibility with task switching, attention and concentration [32]. Processing speed is associated with increased capacity for working memory, enhanced inductive reasoning and greater accuracy in solving arithmetic word problems, and consistently predicts performance on cognitive tasks [16].

Table 2 summarizes studies that explore the impact of HIV on important neurocognitive domains. PHIV+ children have been found to perform significantly poorer in EF tasks, particularly in terms of processing speed [13,14,26,33], memory [12,14,21,24,34] and attention [13]. Lower scores on visual–spatial processing have also been described in younger PHIV+ children [27,35]. Visual–spatial processing is important for adolescents as it impacts on reading, writing and learning. PHIV+ children have been shown to be slower and less accurate on pattern recognition [26], and to have lower scores than controls on sequential processing, simultaneous processing [36], planning/reasoning [13] and visual memory [24].

Adaptive functioning

Adaptive functioning has been defined as the ability to function effectively in a number of settings requiring social and problem solving skills, including school, home and social settings [37]. Cognitive assessments may not be the appropriate measurement tools to capture the ability of children and adolescents to function in real life situations. For example, in child-headed households in resource-constrained settings, children are required to take far more responsibility than in resource-rich countries. Measuring adaptive functioning, as previously used in younger children, may provide a more meaningful way of assessing how adolescents are functioning in their own environments. There is conflicting evidence on the correlation of scores. Gosling *et al*. found significant weakness in adaptive functioning compared with cognitive functioning in PHIV+ children [38]. In contrast, Smith *et al*. found some disparity, with higher scores in adaptive function at lower cognitive scores [14]. As the number of PHIV+ children grow, further research on this is needed to determine whether measuring adaptive functioning is a useful measurement tool for neurodevelopmental outcomes in PHIV+ adolescents in less developed settings.

The interplay between HIV, neurodevelopment, behaviour and mental health

Several studies have focused on the burden of psychiatric problems and mental health functioning impairment in PHIV+ children and the interplay with EF, risk-taking behaviour and treatment adherence.

A study in the United States observed a 25% prevalence of mental health problems among PHIV+ children and adolescents, well above that of the general population though lower than the 38% rate observed in the PHEU comparison group [39]. Caregiver characteristics (psychiatric disorder, limit-setting problems and health-related functional limitations) and child characteristics (younger age and lower IQ) were most predictive of the occurrence of mental health problems. Another US study documented that 18% of 6–17 year old PHIV+ children had a lifetime history of psychiatric medications, 13% were on medication (largely stimulants and antidepressants) for psychiatric problems and 22% had a past or current history of non-medication psychological intervention [40].

There is a strong association between psychological and neurocognitive functioning. In a study in Atlanta and New York City, depressive symptoms in PHIV+ adolescents were best predicted by a combination of negative coping skills and poor neuropsychological functioning. Conduct disorder problems were directly associated with neuropsychological functioning (cognitive inflexibility) and negative coping skills [41]. A study in New Zealand reported that risky personality and performance on the neuropsychological and EF tests were significant predictors of risk-taking [42]. Furthermore, psychiatric disorders and behavioural health challenges in PHIV+ children can lead to poor ART adherence, risk-taking behaviour, including risky sexual behaviour, precocious sexual debut, teenage pregnancy and substance abuse [40,43–48].

These findings add weight to the increasing concern about long-term neurodevelopmental problems among PHIV+ adolescents [8,49] and the burden that these pose for individuals, families and the education and health care systems.

Language and hearing

As children transition to early and middle adolescence, language and reading skills are the critical building blocks for literacy and future academic success, with an important transition from ''learning to read and reading to learn'' [50]. There is evidence that verbal skills are negatively affected in PHIV+ children [14,24,36,50,51]. In a large study in New York City, vocabulary and reading were worse in PHIV+ youths compared to PHEU, even after adjusting for demographic variables [50]. In contrast, Rice *et al*. in a multisite US (including Puerto Rico) study found that both PHIV+ and PHEU performed poorly on verbal tests, but there was no difference between the two groups [51].

Table 2. Specific neurocognitive domains affected in perinatally HIV-infected children

Study	Participants	Age (range)	Measure	Findings
Processing speed:				
Koekkoek *et al.* 2008 [26] The Netherlands	22 PHIV+	Median 9.5 yrs (6–13.5)	Amsterdam neuro-psychological task: baseline speed	Significantly slower compared to age-appropriate norms
Smith *et al.* 2012 [14] USA+ Puerto Rico	88 PHIV+/C 270 PHIV+/NoC 200 PHEU	7–16 years	WISC-IV Processing speed	Lower scores on processing speed for PHIV+/C compared to PHIV+/NoC and PHEU. PHIV+/NoC and PHEU scores were similar
Nachman *et al.* 2012 [34] USA	319 PHIV+ IQ > 70	6–17 years	WISC-IV coding recall	Higher peak viral load (> 100 000 copies/ml)and lower nadir CD4% (< 15%) associated with slower speed
Ruel *et al.* 2012 Uganda [13]	93 PHIV+ 106 PHU CD4 ≥ 15% CD4 count ≥ 350 cells/μl	Median 8.7 yrs (6–12)	Test of variables of attention	Worse visual, auditory and overall reaction time than HIV-community age matched
Set Shifting:				
Koekkoek *et al.* 2008 [26] The Netherlands	22 PHIV+	Median 9.5 yrs (6–13.5)	Amsterdam Neuro-psychological task: Attentional flexibility	Significantly slower compared to age-appropriate norms Better outcomes with longer HAART duration
Verbal Fluency: (EF in the verbal domain)				
Koekkoek *et al.* 2008 [26] The Netherlands	22 PHIV+	Median 9.5 yrs (6–13.5)	Verbal fluency	Significantly lower scores compared to age appropriate norms
Hoare *et al.* 2012 [24] South Africa	12 PHIV+ 12 HIV −	Mean 10.4 yrs (8–12)	Semantic fluency	Significantly lower than HIV-negative controls from same neighbourhood
Memory				
Blanchette *et al.* 2002 [27] Canada	14 PHIV+ 11 control siblings	6.3–14.9 yrs	WISC-digit span and information Story recall Rey Complex figure	No difference between groups
Bagenda 2006 Uganda [12]	28 HIV+ 42 HEU 37 HIV −	6–12 years	KABC Sequential processing (Immediate memory recall)	HIV+ significantly lower scores than HEU No difference between PHIV+ and HIV- groups
Martin *et al.* 2006 [21] USA	41 PHIV+	Mean 11.2 yrs (6–16)	WISC III – working memory: Digit span backwards Arithmetic	Significantly lower scores in those with abnormal CT brain scans compared to those with normal scans

Table 2 (*Continued*)

Study	Participants	Age (range)	Measure	Findings
Hoare *et al.* 2012 [24] South Africa	12 PHIV+ 12 HIV−	Mean 10.4 yrs (8–12)	Working memory: WISC IV digit span Backward Visual memory: Rey complex figure	Groups performed similar for working memory Visual memory significantly worse in PHIV+ compared to HIV-controls
Smith *et al.* 2012 USA+Puerto Rico [14]	270 PHIV+/noC 88 PHIV+/C 200 PHEU	7–16 years	WISC IV: Working memory	2 to 5 fold increased risk of impairment for HIV+/C group compared to PHEU group
Visual spatial memory/integration				
Koekkoek *et al.* 2008 [26] The Netherlands	22 PHIV+	Median 9.5 yrs (6–13.5)	Amsterdam neuro-psychological task: visuospatial memory	Significantly lower scores in visuospatial working memory compared to age-appropriate norms.
Puthanakit *et al.* 2013 [29] Thailand, Cambodia	284 PHIV+, 155 PHEU, 164 PHU	Median 9 yrs (1–12),	Beery Visual Motor Integration	No difference between early and deferred ART initiation RCT arms PHIV+ children performance worse than PHEU and PHU
Hoare *et al.* 2012 [24] South Africa	12 PHIV+ 12 HIV−	Mean 10.4 yrs (8–12)	Spatial processing: WASI block design Rey complex figure test	Significantly worse than HIV-negative controls

PHIV+/C: Perinatally HIV-infected with a previous class C event.
PHIV+/noC: Perinatally HIV-infected with no past history of class C event.

While CD4 cell count, HIV viral load and CDC Classification were not associated with verbal scores in the New York City study [50], two other US studies found that a history of an AIDS-defining illness was associated with verbal comprehension impairment [14,51]. In addition, Rice *et al*. found that after controlling for cognitive and hearing impairment, children who were PHIV+ with detectable viral load and ART initiation less than six months of age had an increased risk of language impairment. Other risk factors for language impairment combined with cognitive or hearing impairment were race/ethnicity, caregiver's education and intelligence quotient (IQ) status and having a non-biological parent as caregiver [51].

Adjustment for hearing deficits in language assessment of PHIV+ children is important as the prevalence of hearing loss in PHIV+ children is high and ranges from 20% in higher income countries to 38% in low-resource settings [52,53]. In resource-poor settings, hearing loss was largely conductive, including chronic suppurative otitis media and dry tympanic membrane perforations, which may reflect the lack of consistent otological care, whereas in well-resourced settings more children had sensorineural hearing loss [54], which may possibly be related to measurement in the United States. A low CD4 count and a history of AIDS-defining illness were associated with both hearing and language impairment [53,54].

School performance

School performance is a functional outcome that is highly relevant in terms of future quality of life and employment prospects [55]. Academic failure predicts problems in schooling and leads to an increase in school dropouts [56]. Insight into the school performance of PHIV+ children is important in order to plan appropriate resources to support this vulnerable population. However, accurate measurement is problematic due to the abundance of potential confounders. A child's school performance is dependent on numerous variables including social and family factors [55,56]. In addition, the indirect effects of HIV infection including hearing loss, school absenteeism due to ill health or ART management, depression and/or social problems need to be considered when interpreting school performance [33].

Several studies have explored school performance among PHIV+ children and adolescents, and identified poorer outcomes compared with children without HIV, with the exception of a French study, which reported an academic failure rate of 16%, similar to the general population [57]. Outcome measurements were highly variable and included 42% with a learning disability [25], 27–33% receiving special education [50,57,58], 15% having repeated two or more grades [57] and 51% having failed at least one grade [59]. Limited caregiver education or intelligence level increased the risk of poor educational outcomes [14]. There is a striking lack of studies on academic achievement in resource-limited countries. Although such research would be difficult to undertake, it would provide valuable information to guide interventions.

Physical disabilities due to neurological problems

Physical problems due to HIV encephalopathy have been well described in the pre-ART era [10]. There is however a paucity of data on neurological outcomes of ART-naïve PHIV+ child non-progressors as well as those on ART, particularly in older children. In Uganda, Bagenda *et al*. describe children with hypotonia, hyperreflexia and delayed milestones, which disappeared as they grew older [12]. Boivin *et al*. also found motor impairment in PHIV+ asymptomatic children in the first two years of life and later in childhood (ages 8–12 years) [36].

Two South African studies described motor deficits and neurological manifestations in PHIV+ children [9,60]. Govender *et al*. reported 59% abnormal neurological examinations in children aged one month to 12 years, 41% with global pyramidal long tract signs and 16% with cortical visual impairment. However, there were many participants with neurological sequelae due to secondary infections and the direct effects of HIV infection are not clear [60]. Smith *et al*. found evidence of motor dysfunction in 33% of ART-naïve children with no improvement after six months of treatment [9]. In a cohort of 210 PHIV+ French children followed since birth, at a median age of 15 years, three children had persistent motor dysfunction and five had mild to moderate physical impairment, indicating a low incidence of physical disabilities due to neurological problems in children who gain timely access to ART [57]. Some of the neurological manifestations in the young child may not be reversible and may still be evident in the adolescent. We have included these studies to emphasize that a neurological examination should be included when measuring functional outcomes in PHIV+ adolescents.

CNS disease and stroke have been documented as causes of death in PHIV+ children, adolescents and young adults in the USA [8]. In the pre-ART era, the annual risk of cerebrovascular events was 1.3% [61], but there are no data on the incidence of stroke in PHIV+ children on ART. Similarly, the incidence and effect of central nervous system insults caused by infections such as tuberculosis and meningitis have not been well documented.

Markers of HIV disease progression and severity

Traditional markers of HIV disease progression and severity including high plasma viral load, lower CD4 cell counts and/or CD4%, and history of an AIDS defining illness have been associated with poorer neurocognitive performance [13,14,21]. In addition, some markers of vascular dysfunction and T-cell activation have been found to correlate with global cognitive outcomes in PHIV+ youth. Specifically, higher soluble P-selectin, a marker and mediator for inflammatory vascular disease, and lower fibrinogen (a pro-coagulant state marker) have been associated with poorer cognitive function [62]. $CD4^+$ activation and, under certain circumstances, $CD8^+$ activation have been shown to have favourable neurodevelopmental implications in PHIV-infected children [63]. In a study of ART-naive Ugandan children, HIV subtype-A was associated with higher viral loads and poorer

performance compared to subtype-D, suggesting that subtype-A may be more neuropathogenic in children [64].

Intervention strategies

Studies have found that ART alone is not sufficient to reverse the neurodevelopmental consequences of HIV infection [31,65]. Highly active ART (HAART) may even contribute to neuromotor decline over time [31,66]. The inability of ART alone to restore HIV+ children to ''normal'' neuropsychological performance is a compelling rationale to evaluate alternative interventions for neurocognitive disability in paediatric HIV. Despite knowledge of deficits in PHIV+ children, there have been very few intervention studies. One intervention is computerized cognitive rehabilitation training [67,68]. Preliminary results in Uganda indicate that using computer games for cognitive rehabilitation can be of great benefit to PHIV+ children and adolescents [69]. For younger children with HIV, caregiver training on practical strategies to enrich the developmental milieu of these children can also have significant neurocognitive benefit [70].

There is evidence to suggest a strong link between psychological well-being and the immunological impact of disease progression [71]. HIV-infected children who exhibited signs of resilience tended to have better neurodevelopmental functioning, social–emotional and gross motor functioning [72]. Some approaches to fostering resilience in PHIV+ children have centred around family dynamics within a cultural framework [73–76]. In a qualitative study of resilience among Rwandan HIV-affected children and families [74], Betancourt and colleagues identified five factors that increased resilience in children and families affected by HIV: perseverance, self-esteem/self-confidence, family unity/trust, good parenting, and collective/communal support. Interventions and strategies to leverage these resources may help to prevent mental health problems in these children as they grow into adolescence and adulthood [73]. Psychosocial intervention may also significantly enhance subsequent neurocognitive development of the child in response to the direct physiological, psychological, social and immunological impacts of this disease. For example, Coscia *et al*. showed that home environment had a stronger association with child IQ during the advanced than the early stages of disease [77]. Parental support has been shown to provide a stress-buffering effect for the effects of depression in these school children, that seemed to improve psychosocial and cognitive development [78,79].

Discussion

Each child has their own set of unique factors that shapes their development, making it difficult to identify the relative contribution of different factors impacting on the neurodevelopmental outcomes of PHIV+ adolescents. While HIV has a direct effect on neurocognitive development, the effects of deprivation and poverty, quality of home environment, genetics, opportunistic infections, and access to care may overshadow the effects of HIV, particularly in resource-constrained settings.

Important variables that have been shown to affect neurodevelopmental outcomes include caregiver mental health or substance problems [14], orphan status and chronic illness [60], nutritional status [60,80] formal education and home environment [80] as well as having a biological parent as caregiver, higher family income level and higher caregiver cognitive functioning [14]. Given the psychosocial impact of diagnosis and treatment, as well as the contribution of coping with cognitive weaknesses, additional attention to behavioural and mood symptoms associated with childhood HIV is essential.

It is possible that ART initiation in school-aged children and adolescents may be too late to reverse impairment. Cohorts initiating ART earlier report better outcomes, suggesting that earlier ART initiation is beneficial [6,29]. However, there is inadequate evidence of the effects of long-term ART on the developing brain. Lower nadir CD4 counts, higher viral loads and the history of an AIDS-defining illness are associated with poorer neurodevelopmental outcomes, further supporting the need for early ART initiation in children. Children presenting with these risk factors should be offered neurodevelopmental screening as part of routine HIV care and referral to supportive services or formal assessments where appropriate. PHIV+ adolescents should be provided with multidisciplinary support services including adherence support, reproductive health counselling and mental health and educational/vocational planning [81]. Preliminary evaluations of these multi-faceted interventions for PHIV+ adolescents have shown good results in improving adherence and reducing risk-taking behaviours [81–84].

While most studies describe the proportions of male and female study participants, generally the data were not analysed and compared for sex differences in outcomes. This is possibly because it is generally accepted that the neurodevelopment of boys and girls are similar. However, possible sex differences in adolescent neurodevelopmental outcomes require further exploration.

Conclusions

PHIV+ adolescents constitute a large heterogeneous population. Overall, HIV+ children and adolescents have poorer neurodevelopmental outcomes than uninfected peers, particularly those with more advanced HIV disease. There is also emerging evidence that PHIV+ adolescents are especially at risk for poorer psychiatric outcomes and EFs. However, the impact of HIV on the developing adolescent brain is highly complex, influenced by many factors and not well understood. Compounding and contributory factors may include an increased risk of substance use, risky sexual and other risk-taking behaviours, and poorer ART adherence.

A striking finding is the paucity of data specific to the adolescent age group (10–25 years) and the lack of longitudinal cohort studies designed to assess the effect of HIV on neurocognitive functioning in PHIV+ adolescents. While much of the current evidence is from younger ages, evidence from these studies provides valuable information as neurodevelopmental problems occurring at younger ages are likely to persist in adolescence and adulthood. Furthermore, the majority of studies on neurodevelopmental outcomes in adolescents are from the United States and Europe, with few studies from low- and middle-income countries which have

the highest prevalence of PHIV+ adolescents. Few studies explore possible gender differences in adolescent neurodevelopment. Finally, little is known about the complex nature of recovery of the brain after initiation of ART. Thus, there is an urgent need for longitudinal research assessing the long-term effect of ART and timing of ART initiation on neurodevelopmental outcomes of perinatally HIV-infected adolescents by gender, particularly in resource-constrained settings.

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Competing interests
The authors declare that they have no competing interests.

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Chapter 2

Neurodevelopment of infants on early compared to deferred antiretroviral therapy

When this study was designed, the recommendations for starting ART in children infected with HIV was to wait until they were symptomatic.[41]

A small study by Faye et al described benefits of early antiretroviral therapy before 6 months of age in France [25], but there were no studies from high burden settings such as South Africa. There were questions as to whether early ART would be safe – possible toxicity, or whether resistance would develop due to difficulty administering ART in young babies or waning adherence over time.

We assessed the neurodevelopment of infants perinatally infected with HIV enrolled on the Children with HIV Early antiRetroviral treatment (CHER) trial [27, 28] using the Griffiths Mental Development Scales [32] at 10 – 12 months of age. Children starting early ART at a mean of 8.4 weeks of age were compared to those on the delayed treatment arm who started ART at a mean of 31.4 weeks. ART was Zidovudine, Lamivudine and Lopinavir/ritonavir. Uninfected controls from similar neighbourhoods were used as a reference group.

We found that infants initiated on early ART had significantly better locomotor and general GMDS scores than those on deferred ART and were similar to uninfected controls.

This study was one of the earliest providing evidence of the neurodevelopmental benefits of early ART in asymptomatic infants and provided support for the earliest possible diagnosis of HIV and initiation of ART in infants.

This paper has 136 citations (PubMed Sept 2019), including in Nature.[42]

CONCISE COMMUNICATION

Early antiretroviral therapy improves neurodevelopmental outcomes in infants

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Objectives: To evaluate the effect of early versus deferred antiretroviral therapy (ART) on the neurodevelopment of infants from Cape Town participating in the Children with HIV Early Antiretroviral Therapy (CHER) trial.

Design: HIV-infected infants were randomized to early (<3 months) or deferred ART. HIV-uninfected infants (HIV-exposed and HIV-unexposed) provide background data.

Methods: Neurological examination and Griffiths Mental Development Scales (GMDS) were administered between 10–16 months of age by testers blind to HIV status and randomized allocation. Mean quotients were compared using paired Student's *t*-tests.

Results: Sixty-four infants on early ART and 26 on deferred ART (of potential 77 and 38 respectively on CHER trial) were assessed at median age 11 months (range 10–16). On the GMDS, all scores were lower in the deferred arm and the General Griffiths and Locomotor Scores were significantly lower: mean (SD) = 100.1 (13.8) vs. 106.3 (10.6) $P = 0.02$; and 88.9 (16.3) vs. 97.7 (12.5), $P < 0.01$, respectively. Children with HIV who received early ART performed as well as children without HIV except on the Locomotor subscale. Both infected and uninfected mean GMDS scores were within the average range.

Conclusion: Infants initiated on early ART have significantly better Locomotor and general scores on the GMDS at median age 11 months compared to infants on deferred ART, despite careful monitoring and ready access to ART in the latter.

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Keywords: early antiretroviral therapy, HIV, infants, neurodevelopment

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Introduction

The prevalence of neurodevelopmental delay and/or HIV encephalopathy in HIV-infected children from predominantly well resourced countries has been reported to be between 13 and 23% [1]. Studies from resource-limited settings, wherein antiretroviral therapy (ART) is generally started later, have also reported neurodevelopmental delay [2–5]. However, few studies have prospectively evaluated the impact of early ART on the neurodevelopmental outcomes of HIV-infected infants, and there have been no randomized trials evaluating this outcome. Previous studies have frequently included infants exposed perinatally to other risk factors for poor neurodevelopmental outcome such as illicit drugs [6–8].

To determine the effect of early vs. deferred ART on early neurodevelopmental outcomes, we compared the neurodevelopmental profile on the Griffiths Mental Development Scales (GMDS) [9] of HIV-infected infants who started ART before 12 weeks of age with those for whom ART was deferred until they met immunological or clinical criteria. This was a substudy of the Children with HIV Early Antiretroviral Therapy (CHER) trial as previously described among children enrolled from the Cape Town clinical site [10].

Methods

This cross-sectional substudy compared neurodevelopmental outcomes among participants in CHER randomized to early versus deferred ART, and among HIV-uninfected infants (HIV-exposed and HIV-unexposed) participating in a linked vaccine study [11]. Children were eligible for inclusion if they had no dysmorphic syndromes or underlying non-HIV-related central nervous system abnormalities. The uninfected children were recruited from a concurrent study of pneumococcal conjugate vaccine to provide additional data on the GMDS for South African infants from low socioeconomic backgrounds.

In CHER, HIV-infected infants were randomly assigned to three arms: ART deferred until clinical or immunological progression; early ART commenced before 3 months and limited to 40 or 90 weeks. In this substudy, data from the two early ART arms have been combined. First-line ART included three drugs: zidovudine, lamivudine and lopinavir/ritonavir. Second-line ART was didanosine, abacavir and nevirapine.

Baseline viral load, CD4 cell count, time on ART and hospitalization were obtained from the study database. Birth history and maternal education were obtained from the charts and caregiver interviews. Hospital admissions prior to neurodevelopmental assessments were calculated as days in hospital including the days of admission and discharge. Head growth was plotted on Centers for Disease Control and Prevention (CDC) charts.

A neurological examination and the GMDS were performed at the study visit closest to 10–12 months of age by one of four pediatricians blinded to HIV status and randomized allocation. The GMDS 0–2 years was revised and re-standardized on 665 British children in 1996 [9]. The mean (SD) quotients for general quotient and subscales were 100.5 (11.8) and 100 (16), respectively. Significant impairment is regarded as more than 2 SDs below the mean. Quotients on the subscales and the general quotient were obtained from raw scores using data from normal British children. There are five subscales: Locomotor measures the earliest motor milestones; Personal-social assesses early adaptive behaviour using interaction with the environment and skill in dressing and feeding; Hearing and language measures early expressive language and the ability to follow commands and identify objects; Eye and hand coordination measures fine motor and visual abilities; Performance measures fine motor manipulative skill and visual spatial orientation. Standardized instructions, questions and comments were prepared in English, Afrikaans and Xhosa in accordance with the GMDS manual. A single translator assisted all Xhosa-speaking children.

Statistical analysis was performed with Statistica 10 (Statsoft, Tulsa, Oklahoma, USA). Comparisons between groups were performed using either the paired Student's *t*-test or the Mann–Whitney *U* test for continuous variables and the χ^2 and Fishers test for discrete variables or using one-way analysis of variance using the Kruskal–Wallis test. Mean days in hospital was compared using a generalized linear model using the Poisson distribution and log link function. A 95% confidence interval was calculated where applicable and significance was established at *P* value less than 0.05.

The substudy protocol was approved by the Human Research Ethics Committee, Faculty of Health Sciences, Stellenbosch University, registration number N05/05/092. Written consent was obtained from the child's parent or guardian.

Results

A total of 115 HIV-infected infants from Cape Town participated in the parent study, 77 (67%) of whom were randomized to the two early ART arms and 38 to the deferred ART arm. Of these, eight infants died before assessment (all on the deferred arm); 10 (eight on early ART, two on deferred ART) were not enrolled as they

withdrew from the parent study before scheduled neurodevelopmental assessments. Two infants on early ART with underlying neurological disorders (glutaric-aciduria, fetal alcohol syndrome) were excluded. Five infants (three on early ART and two on deferred) were also excluded because they were assessed after the cutoff age (two) or had unreliable scores (two) or repeatedly missed appointment dates (one). Our analysis included 64 infants on early ART and 26 on deferred treatment.

Demographic information is shown in Table 1. HIV-infected infants were predominantly girls and Xhosa-speaking and maternal education was comparable. Birth outcomes were comparable for weight, gestation and mode of birth. Demographics were comparable in the early treatment group and the unexposed uninfected infants, except for language and gestation. There were more Xhosa-speaking infants in the early treatment group (86 vs. 29%) and more infants with gestation more than 37 weeks in the HIV-infected groups. At enrollment into CHER, the treatment groups were comparable on mean absolute CD4 cell count (1746 vs. 2024 cells/μl, $P = 0.5$), CD4% (34.8 vs. 34.9%, $P = 1.0$) and plasma viral load (log10 RNA copies/ml: 5.66 vs. 5.64, $P = 0.8$).

Mean age of starting ART was 31.4 weeks in deferred and 8.4 weeks in early ART ($P < 0.01$). Twenty-four (92%) infants in deferred ART group were on ART at assessments. Mean time on ART before assessments was 18.7 weeks in deferred and 40.9 weeks in early ART ($P < 0.01$). According to the CHER protocol, treatment interruption was planned after 40 weeks of early ART for some participants [10]. Five infants on early ART, whose scores were included, had treatment interrupted (between 1 and 3 months) before neurodevelopmental assessments were performed. One infant, on early ART, was changed to second-line treatment at 6 months, (20 weeks before the GMDS) due to undisclosed treatment interruption by parent. Clinicians assumed treatment failure.

More infants on deferred ART compared with early ART experienced hospital admissions (46 vs. 30%). The deferred group stayed significantly longer in hospital than the early group (mean 9.4 vs. 2.4 days; $P < 0.01$). At assessment, head circumference was similar between the comparison arms.

On the GMDS, all scores were lower in the deferred vs. the early ART group (Table 2). General and Locomotor scores were significantly lower: mean (SD) = 100.1 (13.8) vs. 106.3 (10.6), $P = 0.02$ and 88.9 (16.3) vs. 97.7 (12.5), $P < 0.01$, respectively. All scores in the early ART arm were similar to those in both HIV-uninfected groups, except for Locomotor wherein the HIV-exposed uninfected arm performed better (Table 2).

Discussion

In this study, HIV-infected infants receiving early ART (<3 months old) scored higher on the GMDS scales, particularly on the General and Locomotor scores, compared to those on deferred ART. Children with HIV who received early ART performed as well as children without HIV except on the Locomotor subscale.

Our findings show that children with HIV who start ART earlier have better short-term neurodevelopmental outcomes than infants for whom treatment is deferred. The mean age starting ART in the deferred group was 31.4 weeks. Although this is early compared to many studies describing neurodevelopmental outcomes [3,4,12–15], even this delay is associated with poorer outcomes. The mean time on ART in infants receiving early ART compared to those on deferred ART was significantly longer (18.7 vs. 40.9 weeks), potentially influencing our findings.

Our study is limited by small sample sizes for the deferred ART and uninfected groups. Given the trend to better outcomes for hearing and speech, eye–hand coordination and performance in the group on early ART, larger sample sizes may have provided a more precise estimate. Eight infants in the deferred group died before assessment. Five of these infants had CDC stage C disease. Had they lived to be assessed, they may have lowered the scores in the deferred ART group further, increasing the differences between arms. Literature has established that neurodevelopmental outcomes in children with AIDS-defining illness are worse than in those without such diagnosis [7–8]. There was an imbalance in primary language; there were more Xhosa-speaking infants in the HIV-infected groups than in the uninfected groups. This may indicate differences in cultural and child-rearing practices but our experience of this age group is that early childhood stimulation is similar in the two language groups, as shown in the comparable results. The study is further limited by the cross-sectional nature of neurodevelopmental assessments. Also, viral loads and CD4 cell counts were measured at varying times in relation to neurodevelopmental assessment and ART status, thus not being comparable. We, therefore, cannot comment on the changes over time and response to ART initiation.

The deferred arm had a higher incidence of illness and hospitalization. The degree to which this may have contributed to the neurodevelopmental delay is not clear. More children in the deferred arm than the early arm required hospitalization (46 vs. 30%) and the mean hospital duration for those admitted was 9.4 vs. 2.4 days. This confirms that early ART prevents morbidity, which may have implications for large ART programmes in developing countries.

Table 1. Demographic data of study participants.

	HIV infected deferred ART ($N=26$)	HIV infected early ART ($N=64$)	HIV-exposed uninfected ($N=28$)	HIV-unexposed uninfected ($N=34$)	P-value comparing 4 groups
Sex: male	10 (39%)	28 (44%)	17 (61%)	19 (56%)	0.7
Mean birth weight, g (range)	3019 (2000–3640)	2989 (2188–3790)	3057 (2166–3798)	3139 (2172–4294)	0.8
Gestation					
>37 weeks	24 (96%)	50 (78%)	11 (71%)	21 (62%)	0.2[b]
≤37 weeks	1 (1 unknown)	11 (3 unknown)	17	13	
Mode of birth					
NVD	20 (77%)	55 (86%)	21 (75%)	29 (85%)	0.4
Caesarean section	6	9	7	5	
Mean (SD) head circumference at assessment, centimetres (range)	46 (1.6) (44–52)	46 (1.5) (43–52)	46 (1.5) (42–48)	46 (1.9) (42–50)	0.4
Primary language					
Xhosa	24 (92%)	55 (86%)	19 (68%)	11 (29%)	0.5
Afrikaans	2	9	6	21	
English	0	0	3	2	
Maternal education: median years of formal schooling (range)	11.0 (4–12)	10.0 (1–12)	10.5 (5–12)	10.0 (6–12)	0.4
Mean (SD) absolute CD4 cell count on enrolment to CHER (range)	1746 (582); (746–2926); (*n=24*)	2024 (1065); (358–5255); (*n=63*)	n/a	n/a	0.5
Mean (SD) CD4% on enrolment to CHER (range)	34.8 (7.9); (17.4–51.3); (*n=24*)	34.9 (8.2); (21.6–53.9); (*n=63*)	n/a	n/a	1.0
Mean viral load (log10 RNA copies/ml) on enrolment to CHER (range)	5.66 (0.42); (4.21–5.88)	5.64 (0.41); (3.78–5.88)	n/a	n/a	0.8
Least square mean (SD) age starting ART, weeks (range)	31.4 (16); (8–76)[a]	8.4 (1.6); (6–12)	n/a	n/a	<0.01
Mean (SD) time on ART in weeks (range)	18.7 (12.7); (0–40)	40.9 (5.1); (33–60)	n/a	n/a	<0.01
Hospital admissions: number participants admitted (% of group)	12 (46%)	19 (30%)	5 (18%)	6 (18%)	0.1
Mean days in hospital ± SE (range)	9.4 ± 0.06 (0–87)	2.4 ± 0.08 (0–25)	0.8 ± 1.5 (0–4)	0.9 ± 1.4 (0-6)	<0.01[c]

ART, antiretroviral therapy; CHER, Children with HIV Early Antiretroviral Therapy.
[a]Three participants who started ART after GMDS were included.
[b]Fisher test.
[c]Wald test (Poisson distribution with log link function).

Table 2. Comparison of mean scores and standard deviations on the Griffiths Mental Development Scales.

	HIV infected deferred ART ($N=26$)	HIV infected early ART ($N=64$)	HIV-exposed uninfected ($N=28$)	HIV-unexposed uninfected ($N=34$)	*P*-value comparing 4 groups
Median age, months, (range)	11.1 (10–14)	11.0 (10–16)	11.5 (10–16)	11.5 (10–14)	0.11
General quotient, mean ± 1 SD (range)	100.1 ± 13.8[b] (55–125)	106.3 ± 10.6[c] (71–125)	105.6 ± 9.9 (82–128)	106.9 ± 11.7 (81.0–125)	0.14[#]
Locomotor quotient, mean ± 1 SD (range)	88.9 ± 16.3[a] (48–112)	97.7 ± 12.5[a] (58–124)	105.3 ± 14.1 (68–137)	101.6 ± 3.6 (69–129)	<0.01
Personal-Social quotient, mean ± 1 SD (range)	107.7 ± 15.9 (63–138)	111.3 ± 13.6[a] (79–138)	106.6 ± 12.1 (81–134)	107.4 ± 17.2 (60–136)	0.46
Hearing and speech quotient, mean ± 1 SD (range)	108.4 ± 13.2 (75–131)	112.5 ± 10.4 (85–131)	108.0 ± 13.9 (71–127)	112.3 ± 13.9 (85–139)	0.55
Eye and hand co-ordination quotient, mean ± 1 SD (range)	102.0 ± 16.1 (50–128)	107.4 ± 15.8[a] (67–128)	107.1 ± 10.6 (84–128)	108.8 ± 15.1 (63–133)	0.24
Performance quotient, mean ± 1 SD (range)	95.0 ± 15.9[a] (58–128)	100.3 ± 13.1 (62–128)	99.8 ± 12.7 (63–123)	102.7 ± 15.5 (61–128)	0.16

ART, antiretroviral therapy.
[#]Posthoc deferred ART vs. early ART *P*-value = 0.02.
[a]No data for 1 participant.
[b]No data for 2 participants.
[c]No data for 3 participants.

The GMDS has not been standardized for South African children, but is widely used [16–18] and scores show good correlation with British children from different race and language groups [19]. Our results support the GMDS as an appropriate tool in our setting. The mean scores on all subscales between the unexposed uninfected and the early ART groups were similar (Table 2). Although there is a significant difference in the mean General and Locomotor scores between the early therapy and deferred therapy groups, the means are still within the normal developmental range (within 1 SD) for the GMDS.

Strengths of this study include that this was performed in a setting in which there is limited prenatal recreational drug exposure. The study population is from a poor socioeconomic background, representative of infants accessing public health system and the demographics of HIV infection. Results should be generalizable to the relevant South African population. The study is further strengthened by the inclusion of uninfected groups from similar cultural and socioeconomic backgrounds, which contextualizes the information on performance on the GMDS.

This study provides evidence of the neurodevelopmental benefits of early ART. In infants tested at a median age of 11 months on the GMDS, those initiated at a mean of 8.4 weeks of age had significantly better Locomotor and General scores than when ART was deferred, despite careful monitoring and ready access to ART. It is plausible that the true difference may be larger based on deaths before assessments. In addition, we found little neurodevelopmental difference between infants who received early ART and infants who were uninfected with HIV. These findings support the earliest possible diagnosis of HIV and initiation of ART in infants. However, caution should be exercised in extrapolating to long-term predictions.

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B.L.: Substudy design, assessments of participants, interpretation of data and lead author.

M.C.: Major contribution in producing manuscript.

D.G.: Substudy design, primary statistical analysis.

M.K.: Statistical analysis.

P.E.S.: Substudy design, assessment of participants, assistance with interpretation of results and contribution to manuscript.

E.D.: Investigator on parent study and contribution to manuscript.

A.J.v.R.: Study Coordinator.

A.V.: Parent study design and implementation and contribution to manuscript.

A.G.B.: Parent study design and trial statistician.

S.A.M.: Parent study design and contribution to manuscript.

P.J.P.: Parent study design and contribution to manuscript.

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All authors have read the final text and approved of submission to AIDS. All authors have signed Authorship Responsibility, Financial Disclosure, and Copyright Transfer.

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Conflicts of interest

There are no conflicts of interest.

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Chapter 3

Neurodevelopmental outcome of perinatally HIV-infected children on early limited or deferred continuous antiretroviral therapy at 5 years

After our finding of improved neurodevelopmental outcome on early ART compared to the delayed ART arm in the Children with HIV Early antiRetroviral treatment (CHER) trial, we continued to follow the children through 4 more assessment time points until the age of 5 years, to investigate the effects of planned treatment interruption and cumulative toxicity.

The Griffiths mental development scales (GMDS) were performed at 11, 20, 30, 42 and 60 months [30, 32], and the Beery-Buktenica developmental tests for visual motor integration at 60 months.[33] Children who were HIV-exposed uninfected (HEU) and HIV-unexposed (HU) were enrolled for comparison.

It was challenging to analyse and compare 5 groups, using longitudinal measures and consider missing data. Due to lack of South African norms, the statistical analysis was done using raw scores and quotients. We found no difference between the scoring systems, which contributes to existing knowledge of the performance of South African children on the GMDS.

We found that poorer locomotor and general Griffiths scores at 11 and 20 months of age, in the delayed ART arm, had resolved by 42 months. Neurodevelopmental outcomes at five years in HIV+ children on early time-limited ART were similar to uninfected controls. However HIV+ children scored significantly lower on visual perception test than uninfected controls.[33] This was despite viral suppression and suggested that HIV adversely affected visual perception regardless of ART strategy.

Our findings suggest that limited ART interruption in children perinatally infected with HIV who started ART < 12 weeks of age while asymptomatic, did not negatively affect their neurodevelopmental outcomes at five years, although there was subtle evidence of developmental delay during treatment interruption. This supports clinical, immunological and virological findings from the larger CHER cohort that early time-limited ART appears safe [27].

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RESEARCH ARTICLE

Five year neurodevelopment outcomes of perinatally HIV-infected children on early limited or deferred continuous antiretroviral therapy

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Abstract

Introduction: Early antiretroviral therapy (ART) has improved neurodevelopmental outcomes of HIV-infected (HIV-positive) children; however, little is known about the longer term outcomes in infants commencing early ART or whether temporary ART interruption might have long-term consequences. In the children with HIV early antiretroviral treatment (CHER) trial, HIV-infected infants ≤12 weeks of age with CD4 ≥25% were randomized to deferred ART (ART-Def); immediate time-limited ART for 40 weeks (ART-40W) or 96 weeks (ART-96W). ART was restarted in the time-limited arms for immunologic/clinical progression. Our objective was to compare the neurodevelopmental profiles in all three arms of Cape Town CHER participants.

Methods: A prospective, longitudinal observational study was used. The Griffiths mental development scales (GMDS), which includes six subscales and a global score, were performed at 11, 20, 30, 42 and 60 months, and the Beery-Buktenica developmental tests for visual motor integration at 60 months. HIV-exposed uninfected (HEU) and HIV-unexposed (HU) children were enrolled for comparison. Mixed model repeated measures were used to compare groups over time, using quotients derived from standardized British norms.

Results: In this study, 28 ART-Def, 35 ART-40W, 33 ART-96W CHER children, and 34 HEU and 39 HU controls were enrolled. GMDS scores over five years were similar between the five groups in all subscales except locomotor and general Griffiths (interaction $p < 0.001$ and $p = 0.02$ respectively), driven by early lower scores in the ART-Def arm. At 60 months, scores for all groups were similar in each GMDS scale. However, Beery visual perception scores were significantly lower in HIV-infected children (mean standard scores: 75.8 ART-Def, 79.8 ART-40W, 75.9 ART-96W) *versus* 84.4 in HEU and 90.5 in HU ($p < 0.01$)).

Conclusions: Early locomotor delay in the ART-Def arm resolved by five years. Neurodevelopmental outcomes at five years in HIV-infected children on early time-limited ART were similar to uninfected controls, apart from visual perception where HIV-infected children scored lower. Poorer visual perception performance warrants further investigation.

Keywords: HIV care continuum; Children; ARV; CHER trial; Early time-limited antiretroviral therapy; Neurodevelopment; Treatment interruption; Griffiths mental development scales; Visual perception

Additional Supporting Information may be found online in the Supporting information tab for this article.

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1 | INTRODUCTION

HIV encephalopathy, a common finding in perinatally HIV-infected children, has decreased since the introduction of combination antiretroviral therapy (ART) [1]. Other neurodevelopmental deficits despite ART remain a concern. Although early viral suppression is beneficial for neurodevelopmental outcomes [2-5], the long-term outcomes of children after early ART are unclear and may be compromised by cumulative toxicity or waning adherence. Early treatment with planned interruptions after early virological suppression is a possible solution.

The Children with HIV Early Antiretroviral (CHER) trial conducted in South Africa (2005 to 2011) compared early time-limited ART with deferred ART in asymptomatic HIV-infected infants [6,7]. HIV-infected infants with CD4 ≥25%

were randomized to one of three strategies: (i) ART deferred until indicated (ART-Def), (ii) early limited ART for 40 weeks (ART-40W) and (iii) early limited ART for 96 weeks (ART-96W). Continuous ART was initiated (in ART-Def) and re-initiated (in ART-40W and ART-96W) if CD4% declined <25% in the first year of life and <20% thereafter, or for protocol-defined severe stage B or stage C clinical disease. After a median of 4.8 years, the superiority of early time-limited over deferred continuous ART was confirmed [6].

A neurodevelopmental sub-study of CHER found significantly better locomotor and general development scores at 11 months for early ART, with children in ART-Def scoring significantly lower than the combined early ART arms. However, the early treatment arms had not yet interrupted ART [8]. These neurodevelopmental outcomes contrast with older children from the PREDICT study which showed no difference between early *versus* deferred ART in children with CD4 15% to 24%. In PREDICT, HIV-infected children performed worse than uninfected controls [9].

Here, we present the neurodevelopmental profiles over five years of this CHER sub-study which includes data from the treatment interruption phase. We hypothesized: firstly that the early treatment arms would do better than the deferred arm, and that the improved neurodevelopmental outcomes seen at 11 months in the early treatment arms would persist despite time off therapy, and be similar to HIV-uninfected controls; secondly that within the early treatment groups, carefully guided ART interruption would not affect neurodevelopmental outcomes.

2 | METHODS

2.1 | Study Design and Participants:

This was a prospective, longitudinal observational sub-study of CHER trial, conducted at the Cape Town site only, between 2006 and 2013. Of 411 infants enrolled onto CHER with CD4 ≥25%, 119 HIV-infected infants in Cape Town were available, along with 42 perinatally HIV-exposed uninfected (HEU) and 42 HIV-unexposed (HU) controls from a concurrent linked vaccine study [10]. Inclusion criteria for the sub-study were: birthweight >2000 g, normal neurological examination at a clinical visit near three months of age, no dysmorphic syndromes or central nervous system (CNS) insults, for example foetal alcohol exposure, birth asphyxia or metabolic abnormalities. HEU and HU controls were enrolled at the same time to provide a reference group for interpreting neurodevelopmental scores within the socio-economic and cultural context. HEU infants also controlled for ART exposure to prevent mother to child transmission (PMTCT) and circumstances surrounding growing up with an HIV-infected mother. Mothers or legal guardians were approached from 2006 during a CHER or Vaccine study visit. Written consent was obtained in their preferred language. Participants who missed early enrolment were included at a later visit. The Stellenbosch University Health Research Ethics Committee approved the study, registration: N05/05/092. Due to attrition of controls, additional five year old Xhosa children (7 HEU, 3 HU) were enrolled from the original source communities in 2012. Eligibility criteria included documented evidence of mother's HIV-negative status at birth, child's HIV-1 antibody test negative, birthweight >2000 g, no history of CNS insults, being clinically healthy with normal medical history and general examination performed by a study doctor.

2.2 | Neurodevelopmental Assessments:

The Griffiths Mental Development Scales (GMDS) were performed at a CHER study visit. The baby scales (0 to 2 years) were used at 11 and 20 months [11] and the extended revised version (2 to 8 years) at 30, 42 and 60 months [12]. The GMDS assesses neurodevelopment on six subscales: locomotor, personal-social, hearing and language, eye and hand coordination, and performance (visuospatial skills including speed and precision), adding practical reasoning after 24 months. A general Griffiths score, an average of the subscales, is also obtained.

One of the four GMDS-trained paediatricians conducted the assessments, assisted by a GMDS-trained translator using standardized translations, all blinded to treatment arm allocation. One paediatrician conducted only four assessments, all at 11 months. The other three assessed at all time points. At the beginning of each age time point, the paediatricians assessed a child together and marked independently, and compared scores and discussed discrepancies afterwards. This was repeated on different children until scoring was similar. The GMDS provides standardized norms from typical British children. Quotients were used to compare performance at different time points. The 0 to 2 year scale provides quotients from raw scores, and 2 to 8 year scales provide percentiles and z-scores. For 2 to 5 year olds, we converted raw scores into age equivalents (from the standardized scores 50th percentiles) and calculated a quotient as a percentage of the child's chronological age, for the subscales and general Griffiths. At 60 months of age, the Beery-Buktenica developmental tests of visual motor integration (Beery-VMI), visual perception and motor coordination (sixth edition) were administered [13]. The Beery-VMI measures the ability to coordinate visual perceptual and motor skills by copying geometric forms. Visual perception requires the child to identify matching forms, and motor coordination involves drawing the forms by connecting dots and staying within paths provided, abilities that are not assessed in the GMDS. Standard scores were calculated from raw scores using USA norms. Neurodevelopmental scores are standardized with a mean (SD) quotient or standard score of 100 (15), scores of 90 to 109 are considered average and <70 as intellectual impaired/delayed [11,13].

Neurological examination and head circumference measurement were performed at each visit and the Child Behavior Checklist completed at 20, 42 and 60 months (reported elsewhere) [14]. Parents or caregivers received a written report after each assessment with advice for stimulating weaker areas of development. Children with suspected sensory deficits or significant developmental delay were referred to appropriate diagnostic and therapeutic services. We included assessments previously reported at 11 months [8] to better present the longitudinal developmental profile over five years. Primary endpoints were neurodevelopmental scores at each assessment.

2.3 | Clinical data

On CHER, HIV-infected participants had regular clinical visits: every 4 weeks until week 24, every 8 weeks until week 48 and then every 12 weeks. Assessments included: physical examination, ART adherence and management of any illnesses

or adverse events, and T-cell subsets were done. HIV-RNA viral loads (VL) were only performed if treatment failure was suspected until November 2009, thereafter VL were performed 6 monthly. A blinded independent clinical endpoint review committee adjudicated CDC severe stage B and C events and HIV encephalopathy diagnosis without knowledge of CD4 values, ART status or treatment group [6,7]. HEU and HU children had three monthly clinical assessments for 18 months and then annually thereafter [10].

Demographic and clinical information were obtained from the CHER database. In HIV-infected participants, viral load (VL) and CD4 values from baseline and within six months of the 60 months assessment were used, together with baseline CMV viraemia [15]. HIV-negative status of controls on the vaccine trial was confirmed between 16 and 28 weeks of age [10].

2.4 | Statistical analysis

We used Statistica version 13 (software.dell.com. Dell Inc. 2015). The primary analysis compared neurodevelopmental quotients over time in five groups (three HIV-infected: ART-Def, ART-40W and ART-96W, and two uninfected: HEU, HU) using linear mixed models with group and time as categorical fixed effects, and participants as random effect. We opted for parsimony and therefore compound symmetry was used. Sample size calculations were based on predicted neurodevelopmental outcomes, which we expected to be larger. To detect an average effect size of 7.5 (half SD on GMDS and Beery tests) at 5% significance level, 30 per group would provide 90% power and 25 per group 80% power.

Due to concerns about using British norms for GMDS outcomes, we conducted *post-hoc* analyses using age-adjusted raw scores. Comparison of scores over 5 years was not possible, due to differences in raw score calculation <2 years and >2 years. We therefore compared, time points 1 to 2 and time points 3, 4 and 5 separately.

Summary statistics for background information were compared using chi-squared test for categorical variables and one-way analysis of variance (ANOVA) for continuous measures. Mean (SD) or median [IQR] were reported, depending on normality and homogeneity of variance.

Additional analysis was performed combining the three HIV-infected arms. Spearman correlations were determined between neurodevelopmental outcomes at 60 months and the following: (i) age at starting ART, (ii) time on ART, (iii) time to VL suppression (age at first VL <400 copies/ml) and (iv) CD4 values at enrolment onto CHER. A 95% confidence interval was calculated and significance established at $p < 0.05$. For variables in the mixed model analysis, the Fisher least significant difference was used.

3 | RESULTS

3.1 | Participants

Ninety-six HIV-infected infants of the 119 CHER trial participants in Cape Town were enrolled in the neurodevelopmental sub-study: 28 ART-Def (excluded two refusals and eight deaths before 11 months), 35 ART-40W (excluded four refusals and two to foetal alcohol exposure (FAE)) and 33 ART-96W (excluded four refusals and one glutaric-aciduria, one FAE, one non-adherence to CHER protocol); 63 of 84 uninfected controls from the vaccine study were enrolled; and 10 additional controls at 60 months (total enrolled: 34 HEU, 39 HU). One infant in ART-96W died at 21 months (disseminated tuberculosis) and one HU child died at 48.5 months (motor vehicle accident). At 60 months, retention in arms was 26/28 (93%) in ART-Def, 29/35(83%) ART-40W and 23/33(70%) ART-96W, and retention in controls from baseline was 15/27 (56%) in HEU and 25/36(69%) HU. For details on participants enrolled and assessed at each time point, see Figure S1.

3.2 | Participant Characteristics

There were more boys in the controls than the HIV-infected groups (Table 1). Birthweight was similar between groups. There was variability in the mode of delivery, with vaginal deliveries ranging from 68% (HEU) to 86% (ART-40W). For PMTCT, most mothers and babies received nevirapine and zidovudine. Three HIV-positive mother-child dyads had no PMTCT. The mean age of mothers at birth of HEU infants was slightly older than the other groups (30.9 years *vs.* means between 27 and 28 years). Maternal education level was similar between groups. Almost all HIV-infected infants, but only 36% of HU infants, were Xhosa-speaking. In the HIV-infected groups, baseline CD4 parameters and mean VL were similar while the proportion with CMV viraemia at baseline ranged from 13% to 29%.

Of HIV-infected participants enrolled, those developing CDC stage severe B or stage C diagnosis were 10/28(36%) in ART-Def, 15/35(43%) in ART 40W and 11/33(33%) in ART 96W. These included HIV encephalopathy in four (14%) ART-Def (diagnosed at 5, 9, 31 and 61 months), five (14%) ART-40W infants (at 19, 20, 21, 24 and 31 months) and two (6%) ART-96W (at 10 and 31 months). Table 2 further describes characteristics of HIV-infected participants.

3.3 | Neurodevelopmental assessments and outcomes:

In general, mean GMDS scores declined over time in all subscales and in all groups, including controls. The most significant differences in longitudinal profile between groups were in the locomotor subscale ($p < 0.001$) and general Griffiths scale ($p = 0.02$), driven by initial lower scores in ART-Def arm at 11 and 20 months. The control groups had higher scores than the HIV-infected arms at the first three assessments in most subscales. However, scores for all groups were similar at 42 and 60 months, with mean quotient for each group ranging from 93.2 to 98.7 for the locomotor subscale and from 81.8 to 84.7 for general Griffiths (Figure 1 and Table 3). Not all children were assessed at each time point due to missed visits or withdrawal, mainly relocation to rural areas.

Results for raw scores (controlling for age) were similar to those for quotients. Differences in developmental profile between groups in the locomotor subscale were confirmed between 30, 42 and 60 months (interactive $p = 0.009$), but the general Griffiths was not as robust (interactive = p 0.1). The raw scores also confirmed significant differences in pairwise comparisons between groups in early assessments, and no difference between arms at 5 years, observed for comparing the quotients. Analysis of raw scores in other Subscales

Table 1. Demographic data and clinical characteristics of all study participants

	HIV infected			Uninfected		
	ART-Def (n = 28)	ART-40W (n = 35)	ART-96W (n = 33)	HEU (n = 34)	HU (n = 39)	*p* value
Gender: Male	11 (39%)	15 (43%)	13 (40%)	19 (56%)	23 (59%)	0.29*
Birthweight, g, mean (SD)	3036 (407)	3096 (445)	2912 (411)	3090 (513)	3127 (561)	0.36*
Delivery						
NVD n (%)	20 (71%)	30 (86%)	28 (85%)	23 (68%)	31 (84%)	
C/S n (%)	8 (29%)	5 (14%)	5 (15%)	11 (32%)	6 (16%)	0.23*
Unknown					2	
PMTCT exposure						
Mother n (%)	27 (96%)	31 (89%)	31 (84%)	29 (85%)	–	0.14#
Child n (%)	25 (89%)	32 (91%)	32 (97%)	29 (85%)	–	0.15#
No PMTCT n	0	2	1	0		
Unknown n	0	0	0	5 (15%)		
Mother's age at birth (years) mean (SD)	27.2 (5.1)	27 (4.4)	27.2 (5.8)	30.9 (14.1)	28 (7.2)	0.26*
Mother's education mean (SD) (highest grade attended)	10.0 (2.1)	9.4 (2.3)	9.4 (2.7)	9.9 (2.4)	9.8 (2.0)	0.70*
Language						
Xhosa n (%)	27 (96%)	32 (91%)	30 (91%)	28 (88%)	14 (36%)	<0.001*
Afrikaans n	1	3	3	4	25	
English n				2		
Mean (SD) at baseline						
CD4 count (cells/μl)	1781 (672)	2082 (968)	2076 (1151)	–	–	0.39§
CD4%	35.8 (8.4)	35.5 (8.6)	34.5 (8.6)			0.81§
Viral load at baseline copies/ml Mean (SD)	598,504 (243,254)	528,435 (250,021)	593,933 (244,958)	–	–	0.38§
CMV PCR at baseline n (%) >25 copies/ml	5/25 (20%)	8/28 (29%)	4/30 (13%)	–	–	0.35§

Treatment groups: ART-Def: ART deferred until symptomatic, ART-40W: early ART until 40 weeks then planned interruption, ART-96W: early ART until 96 weeks then planned interruption. Control groups: HEU: HIV-exposed uninfected, HU: HIV-unexposed. Chi-square for categorical variables and ANOVAs for continuous variables
p values: *between 5 groups, #between 4 groups, §between 3 groups.

confirmed no difference in profiles. (See Supplementary material Tables S3, S4 and S5).

For visual perception at 60 months, the HIV-infected groups scored 5 to 9 points lower than HEU group and 10 to 14 points lower than HU, but Beery-VMI and motor coordination were similar (Table 4). During assessments, no children were suspected of having visual problems.

3.4 | Antiretroviral therapy and responses:

Children on early ART began first-line therapy (zidovudine, lamivudine and lopinavir/ritonavir) at a mean±SD of 1.3 ± 0.5 months (ART-40W) and 1.5 ± 0.5 months (ART-96W) compared to ART-Def (6.8 ± 3.5 months; range 3.9 to 17.7) (Table 2). For ART-40W, of 35 enrolled, two left the study before 40 weeks and 4 met criteria for not interrupting; 29/33 (88%) interrupted at a mean age of 11.4 months for a median of 7.0 [IQR 5.0 to 11.0] months. At 60 months, one was still off ART. For ART-96W, of 33 enrolled, three left the study, one died before 96 weeks and nine met criteria for not interrupting; 20/29(69%) interrupted treatment at a mean age of 24.5 months for a median of 8.0 [IQR 7.0 to 36.0] months (one withdrew before restarting). At 60 months, 14 had restarted and five were still off ART. The mean±SD cumulative time on ART until 60 months was similar among ART-40W and ART-96W and shorter than ART-Def. One child changed to second line ART at 2 years due to CD4 <20% (in ART-40W). Table 2 describes the proportion of participants off ART of those assessed at each assessment.

3.5 | Clinical measures at five years

The proportion of infants with severe CDC stage B or C and HIV encephalopathy of those assessed at 5 years is described in Table 2. At 60 months, 92% in ART-Def, 97% in ART-40W and 74% in ART-96W had VL <400 copies/ml. Mean CD4 parameters and mean WHO height-for-age z-scores were similar in the groups.

3.6 | Correlations between clinical parameters and neurodevelopment at five years

Combining all HIV-infected infants, correlations were weak between neurodevelopmental outcomes at five years and age starting ART, baseline CD4 count, time on ART and time to first VL suppression (Spearman r between −0.19 and 0.08).

Table 2. Descriptive characteristics of HIV-infected participants

Antiretroviral therapy for all enrolled participants

	ART-Def N = 28	ART-40W N = 35	ART-96W N = 33	*p* value
Age at ART initiation, months mean (SD)	6.8 (3.5)	1.3 (0.5)	1.5 (0.5)	<0.001
Treatment interruption: n (%)	Not applicable	29/35 (88%)	20/33 (61%)	0.04
Median [IQR] time interrupted (months)		7 [5 to 11][a]	8 [7 to 36][b]	

Assessments at each time in time-limited treatment groups and proportion off ART

	ART 40-W		ART-96W	
Assessment age	Number assessed	Number interrupted (%)	Number assessed	Number interrupted (%)
11 months	34	0	32	0
20 months	33	14 (42%)	29	0
30 months	32	5 (16%)	28	16 (57%)
42 months	29	1 (4%)	26	6 (23%)
60 months	29	1 (4%)	23	5 (22%)

Antiretroviral therapy and clinical parameters at five years for those assessed at 5 years

	ART-Def N = 26	ART-40W N = 29	ART-96W N = 23	*p* value
Cumulative time on ART at 5 years. assessment, (months) mean (SD)	54.6 (3.6)	49.4 (12)	48.1 (14.5)	<0.001
Severe stage B/C diagnosis, n (%)	9 (35%)	13 (45%)	6 (26%)	>0.2[c]
HIV encephalopathy, n (%)	4 (15%)	4 (14%)	2 (9%)	>0.5 [c]
CD4 count mean (SD)	1080 (363)	1192 (492)	1032 (541)	0.45
CD4% mean (SD)	36.6 (5.8)	34.4 (6.9)	33.1 (8.2)	0.22
VL <400 copies/ml	24/26 (92%)	28/29 (97%)[a]	17/23 (74%)[b]	
WHO height-for-age z-score mean (SD)	−0.64 (1.0)	−0.90 (1.2)	−0.83 (0.8)	0.59
WHO weight-for-age z-score mean (SD)	0.09 (1.1)	0.05 (1.1)	−0.26 (0.8)	0.44

Treatment groups: ART-Def: ART deferred until symptomatic, ART-40W: early ART until 40 weeks then planned interruption, ART-96W: early ART until 96 weeks then planned interruption.
[a]Includes 1 who had not yet restarted ART at 60 months.
[b]Includes 5 who had not yet restarted ART at 60 months and one left study before restarting.
[c]Pairwise chi-square comparison between groups.

Higher baseline CD4% correlated significantly with lower locomotor motor scores (r −0.23; $p = 0.03$) and marginally with Beery-VMI (r −0.2; $p = 0.06$) and motor coordination (r −0.1; $p = 0.06$). (Supplementary Material Tables S1 and S2).

4 | DISCUSSION

Through serial testing, we demonstrated poorer locomotor and general Griffiths scores in ART-Def at 11 and 20 months of age, which had resolved by 42 months and was maintained at five years. The limited differences between groups over time on personal-social, language, eye and hand coordination, performance and practical reasoning GMDS subscales is encouraging. Our findings suggest that limited ART interruption under careful clinical guidance in asymptomatic children starting ART ≤12 weeks of age did not negatively affect their neurodevelopmental outcomes at five years. This supports clinical, immunological and virological findings from the larger CHER cohort that early time-limited ART appears safe [6].

At 11 months, neurodevelopmental scores of ART-Def were generally lower than the other arms. Our longer term findings provide new evidence that catch-up/recovery is possible after delayed ART initiation. However, eight early deaths in ART-Def prior to GMDS assessments may have contributed to a survivor effect.

Our finding that all HIV-infected arms had visual perceptual scores significantly below HU children at 5 years, is concerning. Similarly, two HIV-infected arms (ART-Def and ART-40W) also scored significantly below HEU children. This was despite viral suppression and suggests that HIV adversely affects visual perception regardless of ART strategy. However, cultural differences in early childhood stimulation may have influenced these findings as there were more Afrikaans-speaking children

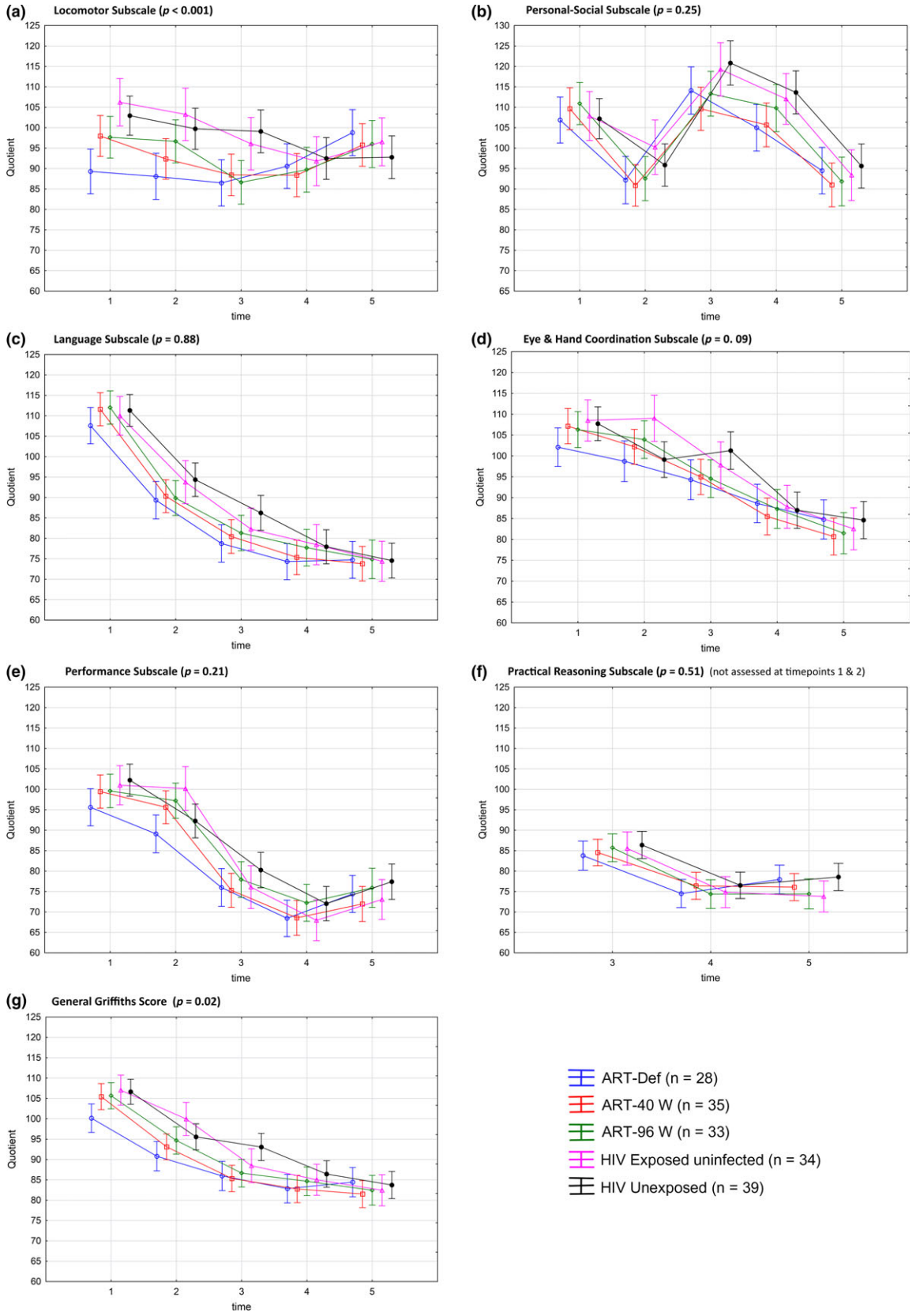

Figure 1. Comparison of Griffiths scales of mental development scores over time per arm. LS means, Type III decomposition, Vertical bars denote 0.95 confidence intervals, interaction p-values. X Axis: Mean age at time points: 1 = 11 months, 2 = 20 months, 3 = 30 months, 4 = 42 months, 5 = 60 months. Note: Lines connecting scores added for visual clarity and do not imply participants identical at each time point.

Table 3. Locomotor subscale and general Griffiths: summary of scores at each time point and comparison between groups

Locomotor subscale: descriptive statistics for each group at each time point

Assessment age	1. ART-Def	2. ART-40W	3. ART-96W	4. HEU	5. HU
11 months	n = 27	n = 32	n = 32	n = 23	n = 35
Mean quotient ±SD	89.5 ± 16.1	98.0 ± 11.8[a]	97.6 ± 14.7[a]	105.9 ± 15.7	102.5 ± 12.9
95% CI	(83.1 to 95.8)	(93.8 to 102.2)	(92.1 to 102.9)	(99.1 to 112.6)	(98.0 to 106.9)
20 months	n = 25	n = 33	n = 29	n = 18	n = 31
Mean quotient ±SD	87.7 ± 18.7	92.6 ± 17.4	97.0 ± 14.0	103.6 ±9.4	99.8 ± 12.3
95% CI	(80.0 to 95.4)	(86.4 to 98.8)	(91.7 to 102.4)	(98.9 to 108.3)	(95.3 to 104.4)
30 months	n = 25	n = 31	n = 28	n = 19	n = 28
Mean quotient ±SD	86.1 ± 16.4	89.2 ± 15.2[a]	87.0 ± 13.3	95.7 ± 14.2[a]	99.2 ± 14.3
95% CI	(79.3 to 92.8)	(83.6 to 94.7)	(81.8 to 92.1)	(88.6 to 102.8)	(93.7 to 104.8)
42 months	n = 27	n = 29	n = 26	n = 21	n = 30
Mean quotient ±SD	90.7 ± 14.5	89.2 ± 14.4[a]	90.1 ± 13.4	91.2 ± 12.8	91.8 ± 16.6
95% CI	(85.0 to 96.4)	(83.6 to 94.7)	(84.7 to 95.5)	(85.3 to 97.0)	(85.6 to 98.0)
60 months	n = 26	n = 29	n = 23	n = 22	n = 28
Mean quotient ±SD	98.7 ± 14.8[a]	96.3 ± 18.0	96.7 ± 13.0	97.1 ± 11.5	93.2 ± 13.2
95% CI	(92.6 to 104.8)	(89.5 to 103.2)	(91.0 to 102.3)	(92.0 to 102.2)	(88.1 to 98.3)

***p*-values for pairwise comparisons between groups for locomotor quotients.**

Assessment age	1 *versus* 2	1 *versus* 3	1 *versus* 4	1 *versus* 5	2 *versus* 3	2 *versus* 4	2 *versus* 5	3 *versus* 4	3 *versus* 5	4 *versus* 5
11 months	0.02*	0.03*	<0.001*	<0.001*	0.9	0.03*	0.15	0.03*	0.14	0.39
20 months	0.26	0.03*	0.001*	0.003*	0.24	0.01*	0.04*	0.12	0.41	0.4
30 months	0.61	0.97	0.03*	0.001*	0.63	0.07	0.004*	0.03*	0.001*	0.47
42 months	0.56	0.82	0.77	0.62	0.73	0.4	0.27	0.61	0.47	0.87
60 months	0.44	0.5	0.59	0.12	0.96	0.85	0.43	0.9	0.42	0.35

General Griffiths scale: descriptive statistics for each group at each time point

Assessment	1. ART-Def	2. ART-40W	3. ART-96W	4. HEU	5. HU
11 months	n = 27	n = 32	n = 32	n = 23	n = 35
Mean quotient ±SD	100.2±13.4	105.5±10.5[b]	105.6±10.2	107.3±10.0	106.5±11.5
95% CI	(94.9 to 105.5)	(101.7 to 109.3)	(101.9 to 109.3)	(103.0 to 111.6)	(102.6 to 110.5)
20 months	n = 25	n = 33	n = 29	n = 18	n = 31
Mean quotient ±SD	90.4±13.6	93.3 to 12.4	94.9±9.1	101.1±8.0	95.6±11.0
95% CI	(84.8 to 96.0)	(88.9 to 97.7)	(91.5 to 98.4)	(97.2 to 105.1)	(91.5 to 99.6)
30 months	n = 25	n = 31	n = 28	n = 19	n = 28
Mean quotient ±SD	85.6±6.0	85.6±7.2[a]	86.7±6.9	89.0±7.5[b]	93.5±9.9
95% CI	(83.1 to 88.1)	(82.9 to 88.2)	(84.1 to 89.4)	(85.2 to 92.9)	(89.6 to 97.3)

Table 3. (*Continued*)

General Griffiths scale: descriptive statistics for each group at each time point										
42 months	n = 27			n = 29		n = 26		n = 21		n = 30
Mean quotient ±SD	83.0±6.9			83.3±5.9[a]		84.7±8.4		84.8±7.8		86.0±10.1
95% CI	(80.2 to 85.7)			(81.0 to 85.6)		(81.4 to 88.1)		(81.2 to 88.4)		(82.3 to 89.8)
60 months	n = 26			n = 29		n = 23		n = 22		n = 28
Mean quotient ±SD	84.3±6.3[a]			81.8±7.0		82.6±6.5		82.8±8.4[a]		84.7±7.7
95% CI	(81.7 to 86.9)			(79.1 to 84.5)		(79.8 to 85.4)		(79.0 to 86.6)		(81.7 to 87.7)
***p*-values for pairwise comparisons between groups for general Griffiths quotients**										
Assessment age	1 *versus* 2	1 *versus* 3	1 *versus* 4	1 *versus* 5	2 *versus* 3	2 *versus* 4	2 *versus* 5	3 *versus* 4	3 *versus* 5	4 *versus* 5
11 months	0.03*	0.02*	0.008*	0.006*	0.92	0.52	0.59	0.59	0.67	0.88
20 months	0.35	0.12	0.001*	0.05	0.5	0.01*	0.28	0.05	0.71	0.1
30 months	0.8	0.78	0.37	0.005*	0.58	0.25	0.001*	0.51	0.01*	0.09
42 months	0.97	0.47	0.41	0.14	0.44	0.38	0.12	0.89	0.47	0.58
60 months	0.24	0.45	0.45	0.78	0.71	0.72	0.35	1.0	0.61	0.61

[a]1 participant and [b]2 participants did not have usable scores (refused too many items or tester error). *Significant differences.

in the HU group. This observation requires further investigation as visual perception may be the underlying cause of deficits found in later childhood. Visual perception involves recognition and discrimination of visual shapes and objects. This process involves the cognitive components and executive tasks of visual attention, memory, discrimination and imagery. Perceptual identification is processed by the ventral stream pathway [16], which may be vulnerable to HIV. Deficits may impair reading, writing and mathematics achievement [17]. Deficits in visual spatial organization, processing and working memory have been described in HIV-infected children, even in the context of normal cognitive development, higher CD4 counts or clinical stability [18-21]. Interestingly, there was no difference between groups on the Beery-VMI and motor coordination tests. Abnormal visual perception in children with normal Beery-VMI scores has been described [17]. It is important to consider that the Beery-VMI may not be sensitive enough to measure the specific visual perceptual deficit caused by HIV in young children.

It is possible that ART interruption at 96 weeks may be better than at 40 weeks, as suggested in the main results paper [6]. There was more HIV encephalopathy in ART-Def and ART-40W compared to ART-96W, and ART-40W also had more CDC severe stage B and stage C diagnoses than the other arms, with the increase occurring largely between the 11 and 18 months' assessments during treatment interruption. On the other hand, fewer children underwent interruption or had baseline CMV viraemia in ART-96W. Interpreting the effects of treatment interruption is difficult as some children randomized to ART-40W or ART-96W were not interrupted, there was a wide range of time off ART, and ART-Def had a longer mean time on ART than the early time-limited arms.

On secondary analysis there were no significant correlations between neurodevelopmental outcome at five years and the following parameters: age at starting ART, time to first viral suppression and time on ART. Interestingly, a higher CD4% at baseline significantly correlated with lower locomotor scores (Spearman r of −0.23) and marginally correlated with lower Beery-VMI and motor coordination scores. Although counter-intuitive, we considered a number of explanations. A higher CD4% in ART-Def may have led to a longer period of observation prior to ART initiation; those on time-limited ART (ART-40W and ART-96W) may have been more likely to interrupt ART than in those who reached an endpoint prior to interruption so would have remained on continuous ART. This issue requires further exploration.

It is interesting to note that on the locomotor subscale, the mean quotients decreased over time in both uninfected groups while increasing in HIV-infected arms. Similar decline over time was noted in most other subscales. The ART-Def and ART-40W arms may be demonstrating catch up due to longer time on suppressive ART from an early age. However, this should be interpreted with caution due to small numbers in infected arms and the enrolment of additional controls at five years.

The GMDS may be insufficiently sensitive to discriminate between the groups at five years, thus creating an impression of "catch up" in the infected groups. Alternatively, the effects of poverty and deprivation on early childhood development may outweigh those of the different ART strategies on neurodevelopment [22,23].

Table 4. Descriptive statistics for scores on the Beery-Buktenica developmental tests for each group

Assessment	1. ART-Def n = 26	2. ART-40W n = 29	3. ART-96W n = 22	4. HEU n = 21	5. HU n = 28	*p* value
Visual motor integration (VMI)						
Mean quotient ±SD	89.7 ± 9.0	89.0 ± 7.3	90.3 ± 11.7	88.0 ± 10.3	92.7 ± 10.5	0.89
95% CI	(86.1 to 93.3)	(86.2 to 91.8)	(85.2 to 95.4)	(83.3 to 92.7)	(88.6 to 96.7)	
Visual perception						
Mean quotient ±SD	75.8 ± 15.9[a]	79.8 ± 14.7[a]	75.9 ± 13.5	84.4 ± 13.5[b]	90.5 ± 9.3	<0.01
95% CI	(69.2 to 82.3)	(74.1 to 85.5)	(69.9 to 81.9)	(77.9 to 90.9)	(86.9 to 94.1)	
Motor coordination						
Mean quotient ±SD	94.1±10.6	93.6±8.4	96.5±7.3	93.9±12.3	92.9±12.3	0.8
95% CI	(89.8 to 98.3)	(90.4 to 96.8)	(93.3 to 99.7)	(88.3 to 99.5)	(88.1 to 97.7)	

***p*-values for pairwise comparisons between groups for Beery-Buktenica developmental tests**

Assessment	1 *versus* 2	1 *versus* 3	1 *versus* 4	1 *versus* 5	2 *versus* 3	2 *versus* 4	2 *versus* 5	3 *versus* 4	3 *versus* 5	4 *versus* 5
Visual motor integration	0.78	0.83	0.56	0.26	0.62	0.73	0.15	0.44	0.39	0.1
Visual perception	0.28	0.98	0.04*	<0.001*	0.31	0.26	0.004*	0.05*	<0.001*	0.13
Motor coordination	0.87	0.42	0.96	0.68	0.33	0.92	0.79	0.41	0.23	0.74

Treatment groups: ART-Def: ART deferred until symptomatic, ART-40W: early ART until 40 weeks then planned interruption, ART-96W: early ART until 96 weeks then planned interruption. Control groups: HEU: HIV-exposed uninfected, HU: HIV-unexposed.
[a]1 participant and [b]2 participants did not complete the test. *Significant differences.

The decline in all GMDS scores except for the personal-social subscale most likely reflects the application of British norms [11,12] to South African children from impoverished environments, and emphasizes the importance of having uninfected controls from the same communities. The GMDS has Xhosa and Afrikaans translations, is widely used in South Africa and thought to be culturally fair [24-26]. However, caution should be used when interpreting these results for clinical purposes. In a *post-hoc* analysis, the longitudinal profiles of age-adjusted raw scores supports our findings when using quotients for locomotor; however, there is a weaker association with general Griffiths and similar scores between groups at five years. We did not see a decline in scores over time for raw scores. (See supplemental material Tables S4 and S5)

The strengths of the study are that this is a relatively cohesive sample recruited from communities with minimal prenatal substance abuse and includes uninfected children from the same communities.. The similar demographic and clinical characteristics in the HIV-infected arms reflect successful randomization of this sub-study within the main trial. A further strength is the longitudinal nature of assessments, despite relatively small numbers and some attrition.

4.1 | Limitations

Our results should be considered within the context of small numbers and variability between groups. The original sample size for this study was calculated assuming a larger difference in neurodevelopmental scores between groups and larger group numbers. Some missed visits and attrition further reduced sample sizes. An inherent bias was that controls had less clinical contact time than HIV-infected children as they had no medication visits, and additional controls were added at five years. Although there were more Afrikaans-speaking HU participants, they were from similar socio-economic backgrounds to the Xhosa-speaking participants with similar neurodevelopmental trajectories. However, we cannot rule out the cultural effects of early childhood rearing practices which may have influenced some comparisons. While we acknowledge that illnesses and maternal depression are important influences on early childhood development, we exclude the effects of hospitalizations and maternal depression due to small sample sizes relative to the number of arms and assessment time points. We have previously described the high incidence of maternal depression and effects of maternal trauma in this cohort [14,27]. As we did not determine whether the HIV infection occurred *in utero* or perinatally, each arm had potential heterogeneity, making further interpretation of ART interruption difficult.

Nevertheless, we found that early ART improved neurodevelopmental outcomes. Planned treatment interruption appeared safe in children who suppressed early. This is reassuring for situations where ART interruption is unavoidable, for example lack of supplies or social/political disruption.

All HIV-infected children should be assessed for visual perceptual deficits and referred for intervention as needed, to improve educational outcomes.

5 | CONCLUSIONS

HIV-infected children on ART-Def arm had locomotor delay at younger ages, which recovered by 5 years. For children with perinatal HIV infection, the neurodevelopmental outcomes at five years of asymptomatic children with preserved CD4 T-cell percentages and receiving early but limited ART under strict

clinical guidance, is similar to HIV-uninfected neighbourhood controls. However, poorer visual perception in HIV-infected children, irrespective of ART strategy, requires further exploration.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

AV, MFC, DMG and AGB designed the CHER trial and provided guidance to BL for design of neurodevelopmental sub-study. BL and PES performed the neurodevelopmental assessments. EFMD provided clinical care to participants, AJvR was the study coordinator, AGB and KO were statisticians for the CHER trial and MK was the statistician for the neurodevelopmental sub-study. BL and MC wrote the first draft of the paper and all co-authors contributed to interpreting findings and writing the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Flow diagram of participants enrolled and assessments performed at each age per group.

Table S1. Correlation between time to first viral suppression (age at first viral load <400 copies/ml) and neurodevelopmental scores at 5 years

Table S2. Correlation between baseline CD4 values and neurodevelopmental scores at 5 years

Table S3. Comparison of statistical analysis using Quotients from British standardized norms and raw scores. Linear mixed model with group and time as categorical fixed effects: interaction *p* value

Table S4. Pairwise comparisons between groups of means for age-adjusted raw scores and quotients in each group for general Griffiths scale

Table S5. Pairwise comparisons between groups of means for age-adjusted raw scores and quotients in each group for locomotor subscale

Supporting information for manuscript: Five year Neurodevelopment Outcomes of perinatally HIV-infected children on early limited or deferred continuous antiretroviral therapy

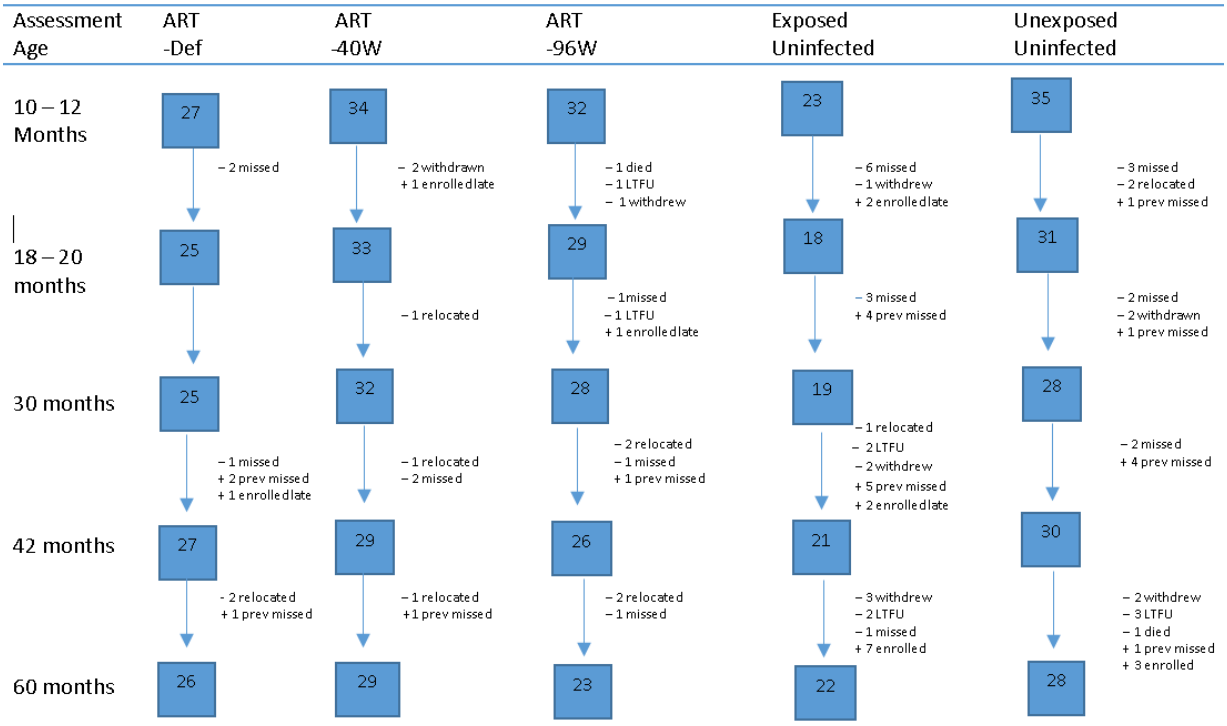

Figure 1: Flow diagram of participants enrolled and assessments performed at each age per group

Correlations between clinical parameters and neurodevelopment at 5 years:

Age starting ART and the General Griffiths quotient at 5 years*:* Spearman r= -0·03

Time spent on ART and the General Griffiths quotient at 5 years: Spearman r=-0·13

Time to first HIV viral suppression and neurodevelopmental scores at 5 years:

Spearman r ranged from -0·12 to 0·07 (table 1)

Baseline CD4 percentage and count and neurodevelopmental scores at 5 years:

Spearman r ranged from -0·23 to 0·08 (table 2)

Table 1: Correlation between time to first viral suppression (age at first viral load <400 copies/ml) and neurodevelopmental scores at 5 years:

	Spearman r	p value
Griffiths Mental Development Scales:		
Locomotor	0.02	0.86
Personal-Social	-0.1	0.4
Language	-0.04	0.74
Eye& Hand Coordination	0.02	0.86
Performance	-0.12	0.29
Practical Reasoning	0.05	0.65
General Griffiths	-0.03	0.82
Beery-Buktenica Tests:		
Visual Motor Integration	-0.06	0.6
Visual Perception	0.04	0.75
Motor Coordination	0.07	0.55

Table 2: Correlation between baseline CD4 values and neurodevelopmental scores at 5 years

	CD4 count		CD4 %	
	Spearman r	p value	Spearman r	p value
Griffiths Mental Development Scales:				
Locomotor	-0.19	0.08	-0.23	0.03
Personal-Social	0.00	0.99	0.08	0.48
Language	0.08	0.48	-0.18	0.11
Eye& Hand Coordination	-0.08	0.48	-0.07	0.52
Performance	-0.03	0.75	-0.10	0.34
Practical Reasoning	-0.09	0.41	-0.03	0.79
General Griffiths	-0.13	0.24	-0.16	0.14
Beery-Buktenica Tests:				
Visual Motor Integration	-0.13	0.25	-0.20	0.06
Visual Perception	-0.19	0.08	-0.07	0.53
Motor Coordination	-0.10	0.37	-0.21	0.06

Comparison of statistical analysis using Quotients from UK standardised norms and raw scores.

Raw scores were adjusted for age at each time point.

The raw scores derived from the Griffiths 0-2 year old (baby scales) are different to the Griffiths 2-5 year old (Extended Revised version) and it was not possible to compare raw scores over 5 time points. (Study objective was to compare neurodevelopmental profiles over 5 year of 5 groups of children). Analysis or raw scores was restricted to comparing the profile in the arms in two sections between time points 1-2 and between time points 3, 4, 5.

Table 3: Comparison of statistical analysis using Quotients from British standardised norms and raw scores. Linear mixed model with group and time as categorical fixed effects: interaction p value

Statistical method used	**Raw scores with age as covariate**		**Quotients**
Time points included	1-2	3,4,5	1,2,3,4,5
General Griffiths	0.84	0.10	0.02*
Locomotor	0.3	0.009	<0.001*
Personal-Social	0.37	0.67	0.25
language	0.15	0.67	0.88
Eye& Hand Co-ordination	0.40	0.46	0.09
Performance	0.40	0.88	0.21
Practical Reasoning	n/a	0.41	§0.51

§ only tested at 3 ,4 ,5

For the General Griffiths raw scores, the differences in neurodevelopmental trajectories in the arms is no longer significant.

The General Griffiths raw score is calculated differently at 1-2 (total of subtest raw scores) and 3, 4, 5 (average of subtest raw scores).

Table 4: Pairwise comparisons between groups of means for age-adjusted raw scores and quotients in each group for the General Griffiths scale.

Time point	Analysis method	ART-Def Vs ART-40W	ART-Def vs ART -96W	ART-Def vs HEU	ART-Def vs HU	ART-40W vs ART-96W	ART-40W vs HEU	ART-40W vs HU	ART-96W vs HEU	ART-96W vs HU	HEU vs HU
1	Raw	0.12	0.07	0.01*	0.04*	0.75	0.23	0.57	0.37	0.82	0.47
	Quotient	0.03*	0.02*	0.008*	0.006*	0.92	0.52	0.59	0.59	0.67	0.88
2	Raw	0.51	0.10	0.00*	0.14	0.27	0.01*	0.36	0.14	0.9	0.1
	Quotient	0.35	0.12	0.001*	0.05*	0.5	0.01*	0.28	0.05*	0.71	0.1
3	Raw			0.47	0.02*	0.85	0.33	0.01*	0.43	0.02*	0.15
	Quotient	0.8	0.78	0.37	0.005*	0.58	0.25	0.001*	0.51	0.01*	0.09
4	Raw	0.90	0.38	0.48	0.11	0.44	0.55	0.13	0.89	0.49	0.42
	Quotient	0.97	0.47	0.41	0.14	0.44	0.38	0.12	0.89	0.47	0.58
5	Raw	0.06	0.26	0.62	0.70	0.7	0.18	0.11	0.55	0.43	0.88
	Quotient	0.24	0.45	0.45	0.78	0.71	0.72	0.35	1	0.61	0.61

For the pairwise comparisons of General Griffiths score, there are discrepancies between the raw scores and quotients. Using raw scores there is no difference between infected groups, but the difference between infected and uninfected groups persist. This is likely due to the effect of locomotor delay being diluted out in the general score. The age adjustment may also have had an effect – quotients are calculated in one month periods – age adjustments were performed on actual age and decimals of months. (i.e children with ages 11,2; 11,4; 11,8 months will all have the same quotient but raw scores are age adjusted) (table 5)

Table 5: Pairwise comparisons between groups of means for age-adjusted raw scores and quotients in each group for the Locomotor subscale.

Time point	Analysis method	ART-Def Vs ART-40W	ART-Def vs ART -96W	ART-Def vs HEU	ART-Def vs HU	ART-40W vs ART-96W	ART-40W vs HEU	ART-40W vs HU	ART-96W vs HEU	ART-96W vs HU	HEU vs HU
1	Raw	0.03*	0.02*	<0.001*	<0.001*	0.8	<0.001*	0.09	0.01*	0.15	0.16
	Quotient	0.02*	0.03*	<0.001*	<0.001*	0.9	0.03*	0.15	0.03*	0.14	0.39
2	Raw	0.47	0.04*	0.02	0.13	0.14	0.06	0.37	0.54	0.63	0.28
	Quotient	0.26	0.03*	0.001*	0.003*	0.24	0.01*	0.04*	0.12	0.41	0.4
3	raw	0.87	0.71	0.18	0.04*	0.58	0.21	0.04*	0.09	0.01*	0.56
	Quotient	0.61	0.97	0.03*	0.001*	0.63	0.07	0.004*	0.03*	0.001*	0.47
4	raw	0.52	0.93	0.89	0.51	0.58	0.45	0.18	0.82	0.46	0.63
	Quotient	0.56	0.82	0.77	0.62	0.73	0.4	0.27	0.61	0.47	0.87
5	Raw	0.35	0.57	0.88	0.06	0.73	0.45	0.33	0.68	0.21	0.1
	Quotient	0.44	0.5	0.59	0.12	0.96	0.85	0.43	0.9	0.42	0.35

Raw score findings for locomotor subscale confirm that scores are similar at 5 years – both raw scores and quotients comparisons at time point are in agreement with no statistically significant differences (apart from two trends).

Chapter 4

Trajectory of clinical signs in children who developed HIV encephalopathy

HIV Encephalopathy (HIVE) is the most severe form of neurological insult in Children perinatally infected with HIV. Since the introduction of ART, the incidence of HIVE has decreased and has changed to a less severe form.[43, 44]

For this longitudinal study of children on the CHER trial, HIVE was recorded as an end point. While conducting neurodevelopmental screening assessments on CHER participants, it was noted that the clinical course was quite variable.

In this chapter we describe the trajectory of clinical signs for those CHER participants who developed HIVE. Possible associations were explored by comparing demographic characteristics of HIVE cases to non-cases.

Interestingly, HIVE was diagnosed when 80% of cases were on ART and 45% had undetectable viral loads for a median [IQR] of 12 [1.4-2.2] years. This has been previously described in smaller studies by Tamula and Innes, the latter described participants on the CHER trial who are also included in this paper.[45, 46] Upper motor neurone signs were the most common (90%), followed by impaired brain growth (80%), gross motor delay (95%) and language delay (60%).

Four children were not on ART, when HIVE was diagnosed, and ART was started. Those who were already on ART at diagnosis continued on their current ART regimen as there was no alternative at that time. UMN signs recovered in 67% over a median of 3.9 years, gross motor delay resolved in 74 % over a median of 1.7 years and language delay resolved over a median of 1.9 years in 92 %. However only 31% of those with impaired head growth reverted to their original growth trajectories.

Our finding prompts further questions about the underlying neuropathological mechanism for prevention and treatment.

This manuscript is considered ready for submission.

Gradual onset and recovery of HIV-related encephalopathy in perinatally infected children after early ART

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Abstract

Background: Although the incidence and severity of HIV encephalopathy (HIVE) in children has decreased with antiretroviral therapy (ART), the effects of early ART on HIVE are not well characterized. We describe the clinical course of HIVE in perinatally-infected children receiving early ART.

Methods: Retrospective case description of participants from Cape Town in the Children with HIV Early Antiretroviral therapy(CHER) trial, who developed HIVE with follow-up for 16 months post-trial.

Results: Twenty(15%) of 133 participants, who initiated ART at a median age 9 weeks, and followed until median age of 6.2 years, developed HIVE. Virological suppression was at median [IQR] of 6[6–19] months later. First deterioration was noticed at a median age of 19 months, when 16(80%) cases were on ART and 9(45%) had undetectable plasma HIV RNA viral load for a median of 12 months. Upper motor neurone signs were present in 90%, of whom 67% recovered over median of 3.9 years and motor delay was present in 95%, of whom 74% recovered over a median of 1.7 years. Four participants had persistent spastic

diparesis. Language delay was present in 60%, of whom 92% recovered over median 1.9 years. Impaired brain growth was present in 80%, of whom 31% recovered over median of 2.0 years. In the 16 participants already on ART at HIVE onset, ART regimens were not altered.

Conclusion: HIV+ children on suppressive ART can develop manifestations of HIVE, the most common being motor deficits and impaired brain growth. However, the majority experienced improvement, with many resolving completely.

Introduction

HIV encephalopathy (HIVE) is the most severe neurological manifestation of HIV in children and can be ameliorated by combination antiretroviral therapy (ART) [1, 2]. The following patterns were initially described for HIVE: static, plateau or sub-acute progressive [3]. However, the manifestations and clinical course are variable, presumably related to individual differences in the underlying timing of infection and mechanism for neuronal insults [4-8]. Early ART initiation dramatically reduces the incidence of HIVE, especially when started in the first few weeks of life [9, 10], however HIVE still occurs [11, 12]. We report the trajectory of clinical signs in a case series of children from a clinical trial who developed HIVE after receiving ART from an early age.

Methods

We reviewed the clinical notes of participants from the Children with HIV Early AntiRetroviral treatment (CHER) trial [13, 14] with confirmed HIVE, and documented the clinical course from earliest deterioration. The CHER trial started in July 2005 and formally ended on 31 August 2011 with participants followed for an additional 14 months through October 2012. In CHER, 377 infants with CD4 ≥25% and below 12 weeks of age were randomized to immediate ART for 40 (ART-40W) or 96 weeks (ART-96W) followed by planned interruption, or ART was deferred (ART-Def) until clinical or immunological deterioration. ART comprised Lopinavir/ritonavir (LPV/r), Zidovudine (ZDV) and Lamivudine (3TC). The Cape Town site contributed 133 (29.5%) participants. Beyond the trial close-out (September 2011), Cape Town participants remained in active follow-up at

the research site. The trial was approved by the Ethics Committees of Stellenbosch University, the University of the Witwatersrand and also the Medicines Control Council of South Africa.

On the CHER trial, participants were reviewed clinically every 4 weeks until week 24, every 8 weeks until week 48 and then every 12 weeks. Review included ART adherence, growth monitoring and a neurological examination. The following developmental milestones were assessed annually: gross motor, language, fine motor and personal-social functioning using the locally-developed Molteno Adapted Scale (MAS) [15, 16]. More frequent neurodevelopmental assessments were performed when an abnormality was suspected.

HIVE diagnosis was determined using the criteria listed below by a blinded end-point review committee (ERC) during the CHER trial and by a team of experts outside of CHER (RvT – pediatric neurologist; and CA – pediatric neuroradiologist) and investigators from CHER (BL, MFC, EFMD and SI) , after the ERC was disbanded. (see figure 1)

Criteria for HIVE were at least two of the following three findings in the absence of an alternative aetiology: (1) Acquired **central motor deficit** manifesting as upper motor neuron (UMN) signs, specifically: pathological reflexes (abnormally brisk reflexes *plus* abnormal spreading of reflexes, crossed adductor response or sustained clonus), increased tone, gait disturbance, ataxia or paresis, with evidence of previously normal neurological examination; (2) **Impaired brain growth** manifesting as acquired microcephaly (reduction in serial head circumference z-score (HCZ) below one standard deviation [SD] from baseline), or generalized brain atrophy demonstrated on radiological imaging; (3) Failure to attain or loss of **developmental milestones** on 2 consecutive visits 3 months apart, using a MAS-derived developmental quotient (developmental age divided by chronological age x 100) <70 in a developmental domain, with evidence of a previously normal quotient. Pre-ART criteria from the CDC required only one of the above three findings for a HIVE diagnosis [17]. However, we used more stringent diagnostic criteria and excluded doubtful or incomplete cases. Alternative aetiologies were excluded after careful clinical, laboratory and radiological investigation. In addition, psychosocial, nutritional or maternal causes were

excluded. We also actively sought to exclude confounders. Social deprivation and lack of stimulation from caregivers is common in our setting and could contribute to neurodevelopmental delay [18]. As poverty, poor education, depression and HIV associated neurological disorder (HAND) may all contribute to mothers not caring adequately for their children, we looked for recent changes in the mothers or their circumstances that might explain developmental delay. Four participants were excluded on this basis.

Where neurodevelopmental milestones were recorded in the absence of a MAS, these additional milestones were retrospectively plotted to obtain a MAS developmental quotient for that individual domain. UMN signs were scored as follows: **0** = Normal; **-1** = Brisk reflexes; **-2** = Extremely brisk or spreading reflexes or increased tone; **-3** = Crossed adductor response; **-4** = Clonus (≥3 beats) or Babinski/up-going plantar response. Gait disturbance related to UMN dysfunction was scored **-5**. UMN function was considered abnormal if UMN score was ≤ **-2**. The presence of brisk reflexes alone was considered insufficient evidence of central motor deficit. We considered a child to have neuromotor deficit when there was a gross motor quotient of <70 and/or UMN score < -2.

World Health Organization growth parameter z-scores for age and gender were used to determine weight-for-age z-score (WAZ), height-for-age z-score (HAZ), weight-for-height z-score (WHZ) and body mass index –for-age z-score (BAZ) and head circumference (HCZ) (WHO AnthroPlus downloadable calculator, https://www.who.int/growthref/tools/en/).

We defined the date of first neurological concern, when developmental quotient <70 or UMN signs (see above) were first documented. The date of nadir for clinical signs was the date of lowest developmental quotient and/or lowest UMN score. The date of first improvement was the earliest date where this was documented. Normalization of development was defined as return of developmental quotient to ≥90. Normalization of central motor deficit was defined as return of UMN score to zero. Time to recovery in each domain was calculated from the first nadir date in that domain.

The onset of acquired microcephaly was defined as the date at which head circumference growth departed from the usual z-score line and progressively dropped by >1 SD. Recovery was defined as return to within 1 SD of baseline head circumference z-score.

HIV RNA PCR viral load testing in plasma was performed on stored specimens from 2005 to 2007 and routinely 3 to 6 monthly after 2007 using a Roche AmpliPrep/Cobas Amplicor assay (Roche Molecular Systems, Pleasanton, California). The lower limit of detection was 400 copies/mL in routine specimens and 40 copies/mL in retrospectively assayed stored specimens. Cumulative time with unsuppressed HIV RNA viral load was calculated with the assumption that, following an undetectable HIV RNA viral load, viral load remained undetectable until the next viral load test.

Cytomegalovirus (CMV) PCR viral load was measured using the Roche COBAS AmpliPrep/COBAS TaqMan (CAP CTM) CMV PCR (Roche Molecular Diagnostics, Branchburg, New Jersey) [19]. Children with positive CMV DNA PCR test in whom CMV viral load was not measureable were assumed to have a CMV viral load equal to the lower limit of detection (150 copies/ml).

Lymphocyte subsets were measured 3 to 6 monthly using a Beckman Coulter single platform lyse no wash procedure using Immunoprep™ reagents with Flow Count™ fluorospheres. Cumulative time with low CD4 absolute count or CD4% prior to recovery was defined as CD4<1000 cells/mm^3 or CD4% <25% for <12 months of age; CD4 <750 cells/mm^3 or CD4% <20% for 12-35months of age; CD4 <500 cells/mm^3 or CD4% <20% for >36months of age.

Statistical Analysis:

Frequencies were determined for categorical variables whereas medians and interquartile ranges (IQR) were calculated for continuous measures. Categorical measures stratified by status of HIVE were compared by the Fishers exact test; continuous measures were compared by the t-test for normally distributed data and the Mann-Whitney test for skewed data. The progression of upper motor neuron signs is presented graphically covering the periods before and after nadir measures. Significance was placed at $p<0.05$. All

statistical analysis was conducted using *Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC.*

Results

Overview of cases

In 133 Cape Town CHER participants followed until median (interquartile range (IQR)) of 6.2 (4.7–6.6) years of age (range 0.1–7.3), ART was initiated at median age (IQR) of 9 (7–12) weeks. Undetectable HIV RNA viral load (VL) was attained at median (IQR) of 6 (6–19) months later. HIVE was recognized in 20 (15%) participants. The origin of cases is depicted in figure 1. Cases are compared to non-cases in table 1. For HIVE, the median (IQR) age when neurological deterioration was first noticed was 19 (16–22) months. At this time 16/20 (80%) cases were on ART of which 9/20 (45%) had undetectable VL for median (IQR) 12 (9–14) months before onset. There were seven on ART with detectable VL. Of the four cases not on ART, three were in interruption phase (all ART-40W) and one was not yet on ART (ART-Def).

UMN signs were present in 18 (90%), of whom 12 recovered fully over median (IQR) 3.9 (2.9–5.0) years, one had partial recovery and four had ongoing spastic diparesis (see table 2). Gross motor delay was present in 19 (95%) of whom 14 recovered fully over median (IQR) 1.7 (1.1–2.6) years, and one was lost to follow up. Language delay was present in 12 (60%) of whom 11 recovered fully over median (IQR) 1.9 (1.4–2.2) years. Impaired brain growth was present in 16 (80%) of whom five recovered over median (IQR) 2.0 (1.6–2.6) years. Progression of neurological deterioration and recovery is presented in table 2. Progression of UMN signs is shown in figure 2 and the gross motor quotient in figure 3 for HIVE cases. The Supplemental Online Figure presents the pattern of language quotient recovery in those affected. Four cases included were previously described [12].

ART regimens were not altered when neurological deterioration was noted as there were no alternatives. By the end of follow-up, five cases had switched from trial-prescribed ART

(ZDV, 3TC and LPV/r): Three switched to Didanosine, Abacavir and Efavirenz or Nevirapine due to assumed virological failure following poor adherence; all later switched back to ZDV, 3TC and LPV/r following viral resistance testing and achieving an undetectable VL after adherence intervention; all three improved neurologically after resuming 1st-line ART and achieving viral suppression. One child was switched from ZDV to Stavudine due to anaemia; he continued to deteriorate neurologically for 10 months after switch and first neurological improvement was noted 17 months after switch. One was switched to Abacavir due to asymptomatic hyperlactatemia discovered after full resolution of gross motor abnormalities; the mild hyperlactatemia subsequently resolved.

Among the 16 cases with impaired brain growth, HCZ did not return to premorbid levels with only five (31%) recovering to within 1 SD of their premorbid HCZ. Median (IQR) HCZ at five years of age was 0.1 (-0.2 to +0.5) SD below their baseline HCZ, however this did not correspond with neurological recovery. Radiological progression of the seven cases with generalized brain atrophy could not be determined as follow-up imaging was not routinely performed. Magnetic resonance imaging (MRI) was performed on 17/20 cases to exclude alternative diagnoses.

CMV was detectable in seven of 18 cases with an available pre-enrolment plasma specimen, of whom only three had a CMV viral load above 150 copies/ml. There was no difference in CMV viral load between cases and non-cases (p=0.64) or in the proportion with detectable CMV between cases and non-cases (p=0.22). One child with HIVE had CMV pneumonia at two months of age with a good response to ganciclovir. Birth weight, nadir WAZ and nadir HCZ were lower in cases than non-cases (table 1).

Discussion

A new pattern of HIV encephalopathy progression and recovery

Unlike previous descriptions of HIVE in the early phases of ART availability, we observed progressive deterioration over 6 to 12 months, followed by gradual recovery as the most common pattern seen. This is in contrast to partial or no recovery. Gross motor and language development recovered over 1 to 2 years and UMN signs resolved over 2½ to 4 years, despite

unchanged ART in 16 cases, however three had persistent spastic diplegia. We previously reported this recovery pattern in four CHER participants who developed stigmata of HIVE which then resolved on continuous ART [12]. Interestingly, 70% did not catch up with head growth and persisted on a lower growth percentile.

The insight that the natural progression of HIVE following early ART is towards recovery is important for interpreting intervention studies to improve HIVE outcomes. This was also described for lower limb muscle tone by Mann et al when reviewing cases referred for a HIVE natural history study by reviewing medical records. Over time, 13 of 19 children identified from a HIVE database had resolved by 2.1 years after the initial visit. All had initiated ART under old guidelines and before early initiation was routine [20]. In our study, neither age at ART initiation nor cumulative time with unsuppressed HIV RNA PCR viral load were significantly different in HIVE cases versus non-cases. The proportion with suppressed HIV RNA viral load at last recorded visit was marginally lower in cases versus non-cases, which may be related to the longer cumulative time on ART in cases due to earlier ART re-initiation following HIVE diagnosis.

Possible mechanism

The pathogenesis of HIVE in virally-suppressed children remains poorly understood. immunopathological processes may be governed by viral and host factors [21]. HIV crosses the blood-brain barrier transported by infected monocytes/macrophages and CD4+ T-lymphocytes. Astrocytes are then infected and a host inflammatory response leads to a neurotoxic cascade that may damage the inflammation-sensitive myelin sheath. While this may occur due to infectious virus, it may also occur in its absence. A correlation has been found between the severity of cognitive impairment and the degree of tumor necrosis factor elevation within astrocytes and microglial cells and the number of activated microglia cells in brain tissue [22], but not with the number of HIV-infected cells or the amount of HIV in brain tissue. Evidence suggests that the pathogenesis of HIVE in children on suppressive ART may be mediated by activated microglial cells and astrocytes over-producing inflammatory cytokines in response to persisting viral proteins, particularly gp120 [2, 23]. Conceivably,

therefore, white matter demyelination that occurs despite virological suppression may be related to such disordered immune regulatory mechanisms. This may plausibly be driven by cytokine release in response to antibodies against myelin oligodentrocyte glycoprotein (MOG). MOG antibodies are implicated in causing neuroinflammation and demyelination in HAND, even after viral clearance [24]. This is consistent with the white matter hyperintensities seen on T2/FLAIR MRI in participants on the CHER study [25] (9 from this cohort were included). Inflammation may have resolved over time as exposure to HIV antigens decreased.

We postulate that initially the brain infection is relatively well controlled, while later, there is a quantitative and/or qualitative breakdown of immune regulation in the CNS. Chronic intrathecal immune activation in HIV+ patients has been reported, even after several years of ART [26]. Markers of intrathecal inflammation include MOG antibodies, myelin basic protein, neopterin, Beta 2-microglobulin, oligoclonal bands and immunoglobulin G index. Notably, ART intensification has no effect on intrathecal immunoactivation, a finding that argues against ongoing viral replication in the CNS. This finding raises the question whether immunomodulatory therapy (at the time of neurological deterioration) may be of benefit; however, that spontaneous recovery occurred in most study participants (albeit at a slow rate) argues against such a need. Another possible contributing factor, that of ART neurotoxicity is unlikely as the children improved on an unchanged regimen. Even though protease inhibitors disrupt astrocyte function at therapeutic concentrations in mouse models, our participants resolved on unchanged LPV/r [27]. We hypothesise that intrathecal inflammation, most likely due to HIV products, contributes to HIVE and that resolution occurs after inflammation has resolved.

The most prevalent HIV-related neurological impairments in the study participants were impaired brain growth and pyramidal tract dysfunction (upper motor neuron signs), which are consistent with cerebral white matter (myelin) involvement i.e. HIV-related leukoencephalopathy. In a neuroimaging DTI study, our group showed predominant involvement of corticospinal tracts in CHER HIV+ children at 5 years of age compared to uninfected controls [28] (only five of the 17who had neuroimaging in this study were included). HIV-associated oligodentrocyte/ myelin injury has been observed clinically from neurological imaging studies and brain biopsies.[29, 30] Myelin injury is also associated with

blood-brain-barrier deregulation. The normalization of brain growth and disappearance of UMN signs in the most children is probably related to cerebral white matter recovery of myelin maintenance (re-myelination) during developmental maturation. Mann et al [20], and our group [31] demonstrated locomotor recovery with time. Whatever the cause, the extent of recovery in our cases suggests that extensive neuronal loss does not occur and, despite visible demyelination, the integrity of neuronal connections is preserved. Further studies are warranted to explore this possibility. The sensitivity of standard CNS imaging for mild demyelination has been shown to be poor, may highlight the need for functional white matter imaging modalities (MR spectroscopy and diffusion tensor imaging) and combining with inflammatory markers in unravelling this disease process.

Limitations

The CHER trial was not a neurodevelopmental trial and focussed on clinical endpoints including mortality. The initial clinical signs may have been subtle in young infants and may not have met objective criteria for a study endpoint. Most participants remained in active follow-up beyond the trial close-out (September 2011), providing additional data on progression of early neurological abnormalities detected during the trial. Unfortunately, we did not have prenatal data including information about maternal viral loads and health status, we also could not determine if HIV infection was pre- or perinatal, which may have contributed to a vulnerable CNS in the developing child.

We used stringent diagnostic criteria for HIVE, requiring two out of three signs (where CDC criteria only requires one), which excluded milder forms of neurocognitive disturbance. The MAS developmental milestone scale is not sensitive enough to detect subtle abnormalities and it is possible that a more detailed developmental assessment tool may have identified additional cases. We did not measure CSF VL and the possibility of low-level CSF viremia (<40 copies/ml) could not be excluded. Only structural imaging was performed and while radiological assessments of brain atrophy were performed by a single neuroradiologist, final assessments of atrophy were subjective. It is possible that volumetric measurements may have identified additional cases.

Conclusion

HIVE may occur after early ART initiation and virological suppression and then resolve on unchanged ART, most likely as intrathecal inflammation subsides.

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Figure 1: Origin of HIVE cases

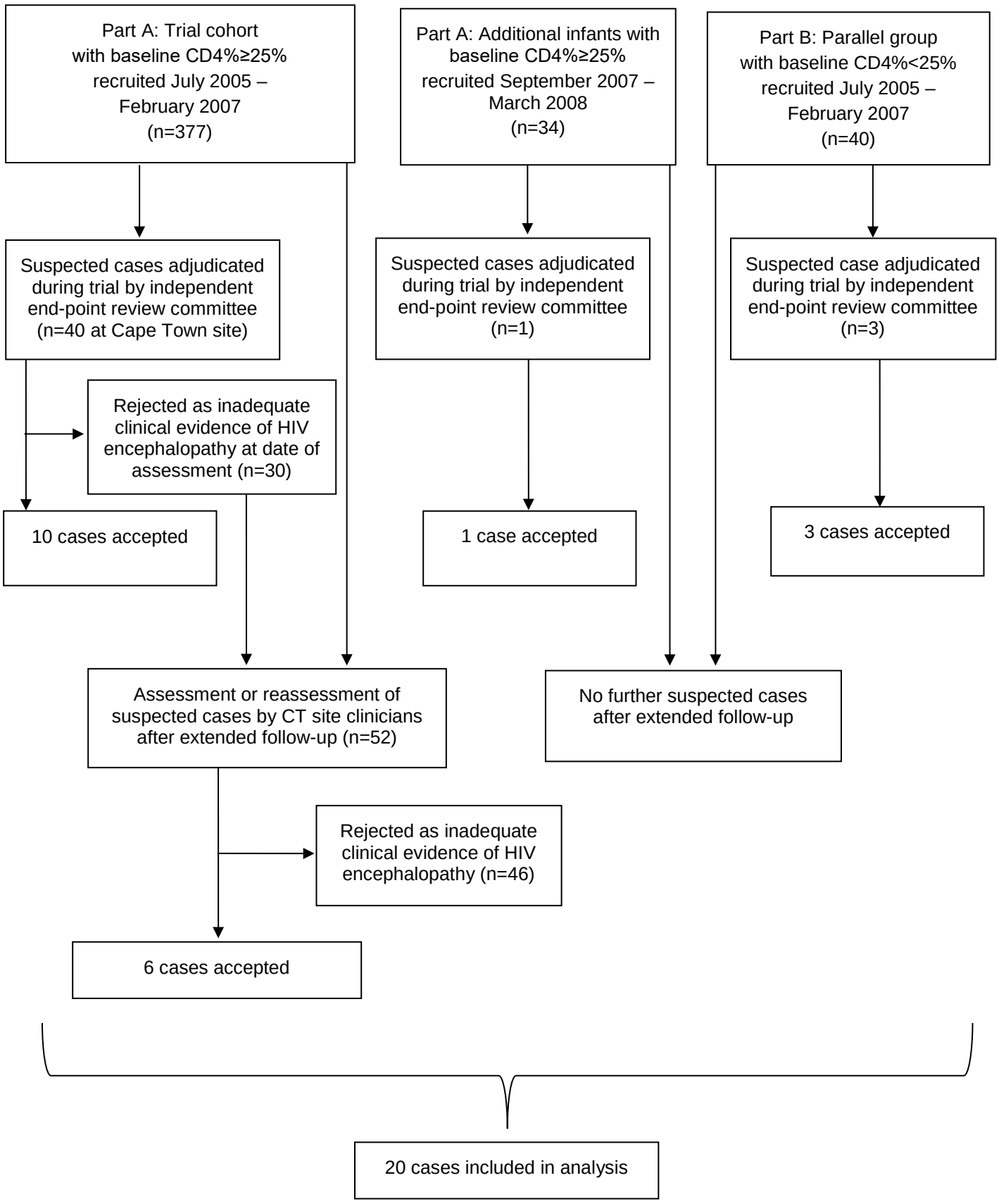


Table 1: Demographics, therapy, immunological status and anthropometry of HIVE cases versus non-cases, presented as median (interquartile range) or number (%) as appropriate

	HIVE Cases n=20	Non-cases n=113	p-value
Gender (male)	8 (40%)	51 (45%)	ns
Receiving nevirapine at birth	17 (85%)	106 (94%)	ns
Receiving zidovudine at birth	18 (90%)	104 (92%)	ns
Birth weight (kg)	2.8 (2.6 – 3.0)	3.0 (2.7 – 3.3)	0.03
Mode of delivery			
• Normal Vertex delivery	17 (85%)	79 (71%)	
• Caesarean Section	3 (15%)	15 (13%)	
• Unknown	0	19 (17%)	
Baseline log_{10} HIV RNA viral load (copies/ml)	5.9 (5.8 – 5.9)	5.9 (5.6 – 5.9)	ns
Cytomegalovirus detectable in pre-enrolment plasma (specimens available for n=118 participants (18 cases, 100 non-cases)	7 (35%) (n= 18)	25 (22%) (n = 100)	ns
Nadir CD4 (cells/mm^3)	688 (446 – 912)	708 (532 – 998)	ns
Nadir CD4%	21% (15 – 25%)	21% (16 – 26%)	ns
Cumulative time with low CD4 or CD4% (months)	5.2 (0.0 – 14.1)	1.5 (0.0 – 8.4)	ns
CHER arm allocation (ART-Def | ART-40W | ART-96W)	5 | 10 | 5	33 | 38 | 42	ns
ART- Def treatment status at onset of HIVE: • Started ART • Not yet started ART	 4 1		
ART-40W treatment status at onset of HIVE: • On ART not yet interrupted • On uninterrupted ART (Site decision to maintain ART) • In protocol-defined interruption phase • Previously interrupted and back on ART for protocol reasons	 0 3 3 4		
ART-96W treatment status at onset: • On ART not yet interrupted • On uninterrupted ART (Site decision to maintain ART) • In protocol-defined interruption phase • Previously interrupted and back on ART for protocol reasons	 2 3 0 0		
Age at first ART initiation (months)	2.2 (1.8 – 2.3)	2.1 (1.7 – 2.7)	ns
Cumulative time on ART (years) §	4.9 (4.8 – 5.2)	4.7 (2.8 – 5.3)	ns
Duration interrupted ART (months) (N=20, of which 9 (<50%) had ART interruption)	0.0 (0.0 – 7.5)	1.8 (0.0 – 7.7)	ns
Cumulative time with unsuppressed HIV RNA PCR viral load (years)	1.7 (1.4 – 3.0)	2.0 (0.9 – 2.8)	ns
Median time in follow-up (years)	6.3 (6.1 – 6.6)	6.2 (2.7 – 6.5)	ns
Virally suppressed at last recorded visit	17 (85%)	73 (64%)	ns
Nadir head circumference-for-age z-score	-0.9 (-1.7 to -0.2)	-0.5 (-1.3 to +0.2)	0.02
Weight-for-age z-score			

At trial entry	-1.0 (-1.9 to -0.7)	-0.9 (-1.8 to 0.0)	ns
Nadir	-2.1 (-3.1 to -1.6)	-1.6 (-2.5 to -0.7)	0.02
Height-for-age z-score			
At trial entry	-1.9 (-2.8 to -1.3)	-1.4 (-2.3 to -0.6)	ns
Nadir	-2.7 (-3.2 to -2.2)	-1.8 (-2.6 to -1.4)	ns
Weight-for-height z-score			
At trial entry	+0.5 (-0.1 to +1.2)	+0.5 (-0.3 to +1.2)	ns
Nadir	-1.2 (-2.7 to -0.4)	-0.6 (-1.8 to +0.1)	ns
Body mass index -for-age z-score			
At trial entry	-0.4 (-1.0 to +0.3)	-0.2 (-1.1 to +0.6)	ns
Nadir	-0.7 (-1.8 to -0.2)	-0.8 (-1.7 to -0.1)	ns
Zenith	+2.1 (+1.2 to +2.4)	+1.7 (+0.9 to +2.7)	ns

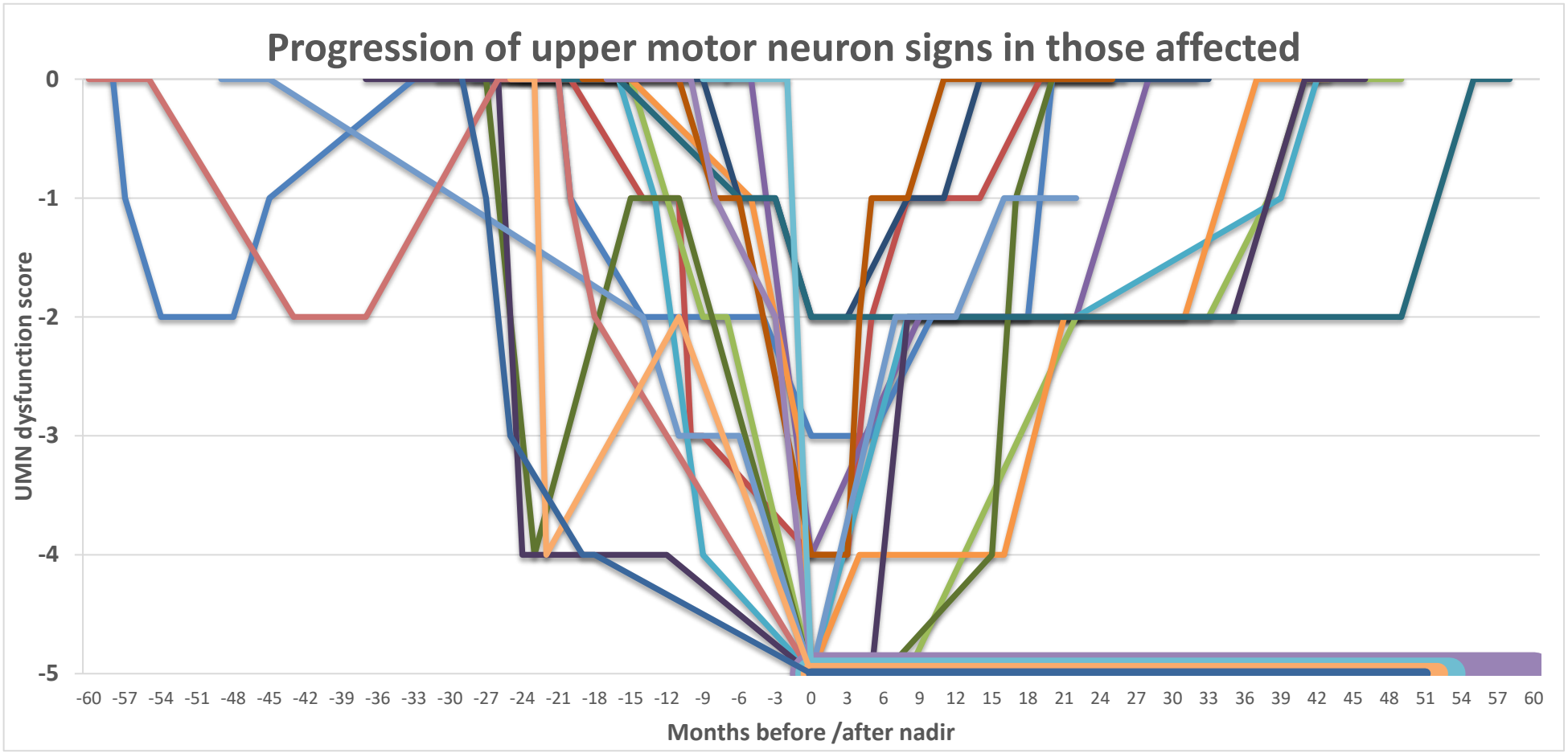
All variables are given as median (interquartile range) unless stated otherwise. Proportions are given as percentages.

Table 2: Neurological deterioration and recovery among HIVE cases (n=20):

	Subgroup	N affected	Full recovery	Partial recovery	Minimal or no recovery	LTFU/No data
UMN	Not on ART	4	3	0	1	0
	ART, VL >1000c/ml	6	2	1	3	0
	VL <400c/ml	8	7	0	0	1 LTFU
Gross Motor	Not on ART	3	2	1	0	0
	ART, VL >1000c/ml	7	4	3	0	0
	VL <400c/ml	9	8	0	0	1 LTFU
Language	Not on ART	2	2	0	0	0
	ART, VL >1000c/ml	6	5	1	0	0
	VL <400c/ml	4	4	0	0	0
Personal Social	Not on ART	1	1	0	0	1 No data
	ART, VL >1000c/ml	3	3	0	0	1 No data
	VL <400c/ml	0	0	0	0	0
Fine Motor	Not on ART	0	0	0	0	1 No data
	ART, VL >1000c/ml	1	1	0	0	1 No data
	VL <400c/ml	0	0	0	0	0
Impaired Brain Growth	Not on ART (2 had imaging)	3 (2 cortical atrophy)	2 (1 cortical atrophy)	0	1 (0 cortical atrophy)	
	ART, VL >1000c/ml (3 had imaging)	4 (0 cortical atrophy)	2 (0 cortical atrophy)	0	2 (0 cortical atrophy)	
	VL <400c/ml (All had imaging)	9 (4 cortical atrophy)	1 (1 cortical atrophy)	0	8 (3 cortical atrophy)	

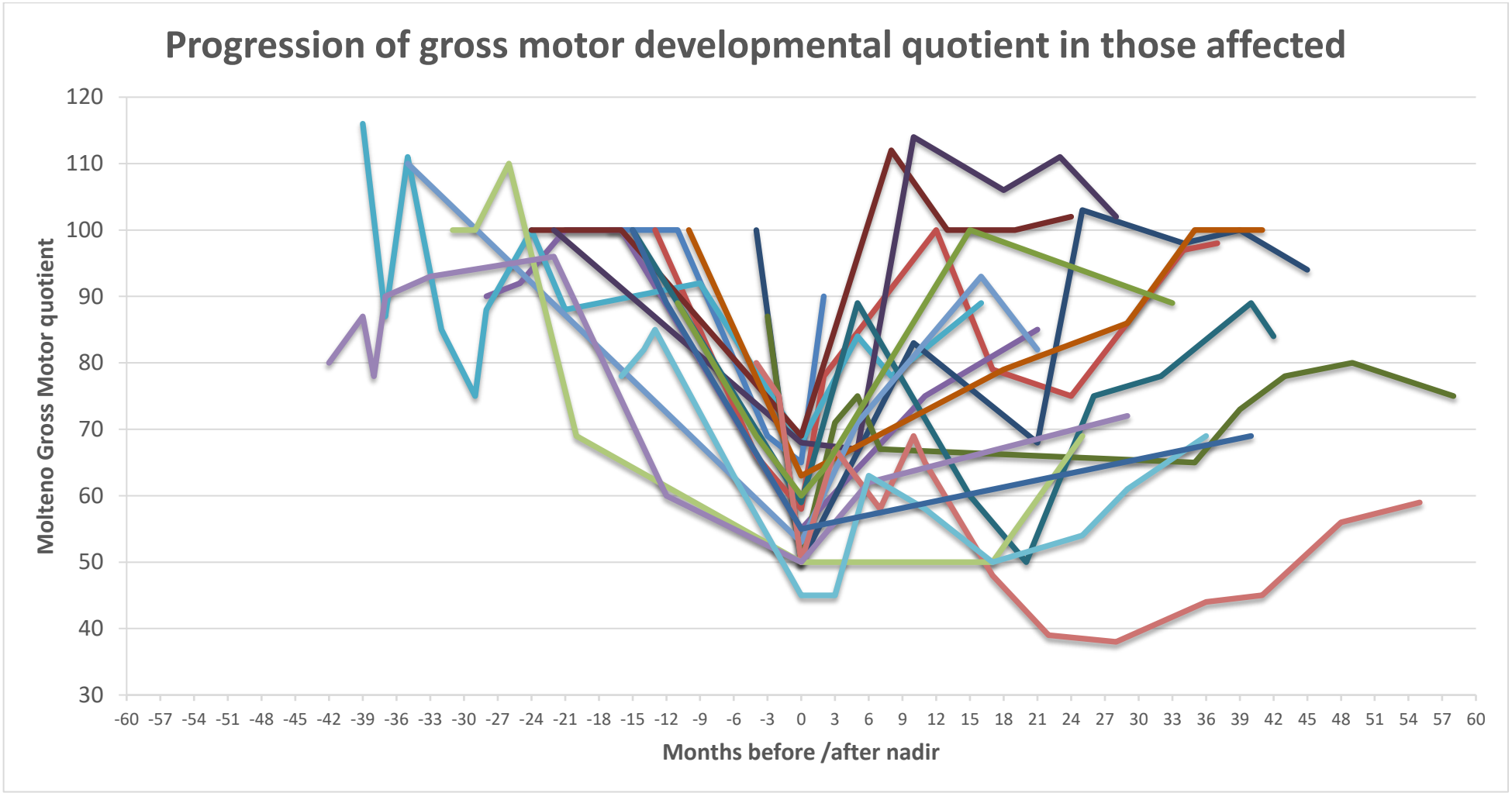
4 cases not on ART at HIVE onset; 7 cases on ART at HIVE onset but viral load >1000c/ml; 9 cases on ART at HIVE onset with viral load <400c/ml.
(Note: no participants had viral load between 400 and 1000c/ml at onset)
LTFU=lost to follow-up. VL=viral load. ART=antiretroviral therapy

Figure 2: Progression of upper motor neuron signs in those affected (n=18)



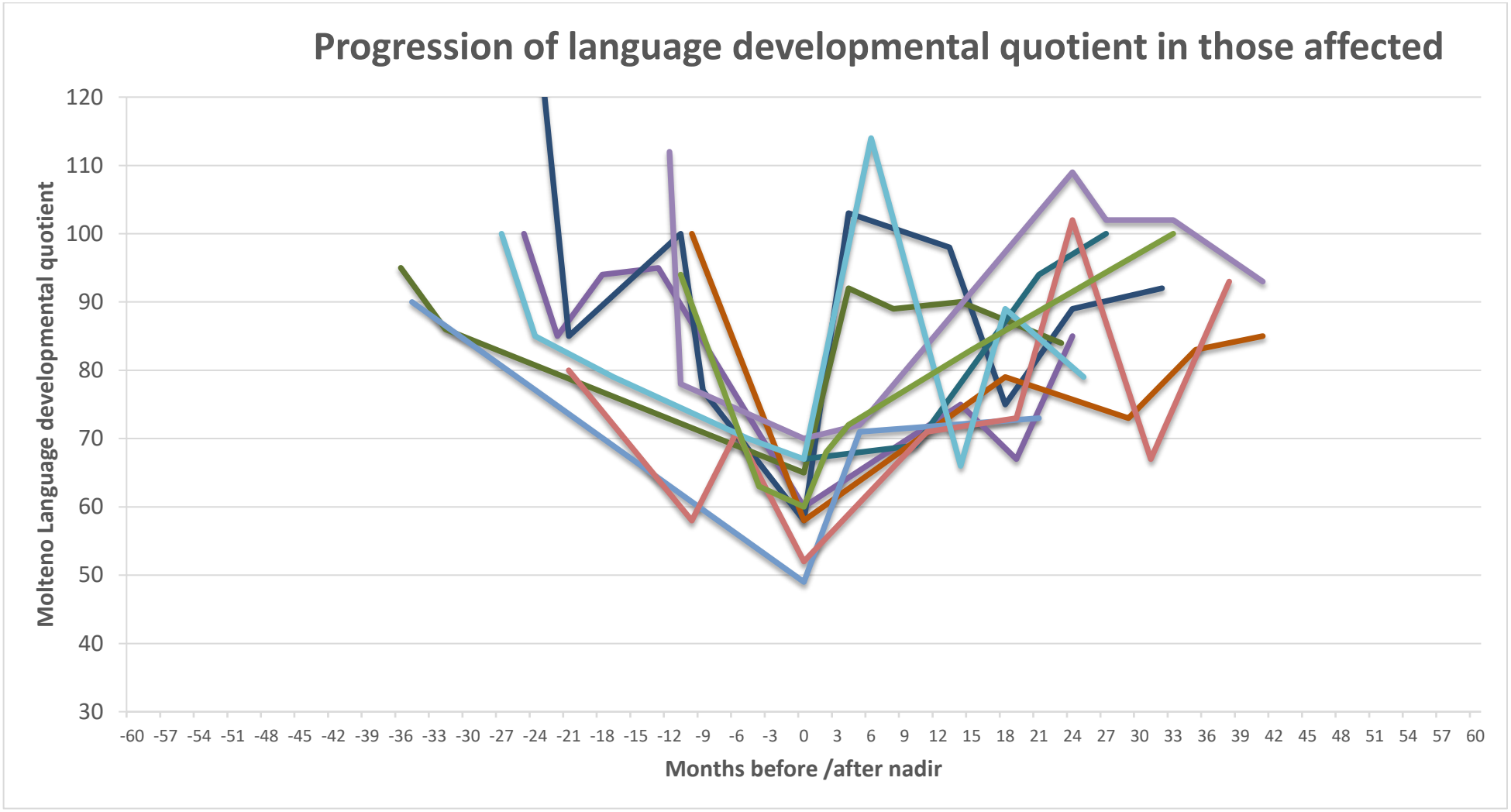
§ Upper motor neuron abnormalities were scored as follows:
0 = Normal; -1 = Brisk reflexes; -2 = Extremely brisk or spreading reflexes or increased tone; -3 = Crossed adductor response; -4 = Clonus (≥3 beats) or Babinski/up-going plantar response; -5 = Gait disturbance related to upper motor neuron dysfunction.
Each line represents an individual case

Figure 3: Progression of gross motor developmental quotient on the Molteno adapted scale in those affected (n=19)



For those with 2 episodes of deterioration, the nadir of the first episode was used as time 0.

Supplemental online figure: Progression of language developmental quotient on the Molteno adapted scale in those affected (n=12)



Chapter 5:

Neurodevelopment after starting antiretroviral therapy within the first few days of life

As knowledge and experience about treating perinatally infected infants improved, it became apparent that early diagnosis and commencing ART as soon as possible after birth may be the best option. This on the premise of decreasing reservoir size and maintaining immune health [39] and the potential for cure or remission.[47, 48] Early diagnosis and treatment programs have been implemented internationally and in South Africa.[40, 49] However neurodevelopmental outcomes from very early ART have not yet been described. Concern about toxicity to the developing brain persists. [50]

This fifth chapter reports on the early neurodevelopmental outcome of infants enrolled onto the very early infant diagnosis program in Cape Town [51, 52] and followed on an NIH funded study (R01MH 105134) to assess the effects of early ART on the HIV reservoir size. ART was Abacavir, Lamivudine and Lopinavir/ritonavir.

Early neurodevelopmental outcomes at 11 months of age for this early treatment cohort (commencing ART at a median of 6 days) are reported as a pilot study (n=29) and compared to CHER participants on early ART arms (commencing ART at a median of 8 weeks of age). Mean scores on the GMDS at 11 months are within the normal range and are similar to scores from the CHER early ART arms, providing no evidence for ART neurotoxicity, after starting very early ART.

There may be a safe window for favourable neurodevelopmental outcome when starting ART before 8 weeks of age. However, the early treatment cohort had more challenges to neurodevelopmental outcome than the CHER cohort (in-utero infection, perinatal infections and more pre-natal drug exposure) and may possibly have scored higher, suggesting that it may be better for neurodevelopmental outcome to start ART as soon after birth as possible.

These should be regarded as preliminary findings in a cohort of only 29 infants.

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Neurodevelopment at 11 months after starting antiretroviral therapy within 3 weeks of life

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Abstract:

Background: Antiretroviral therapy (ART) started between 7 and 12 weeks of age improves neurodevelopmental outcomes in HIV-infected (HIV+) infants, but the impact of even earlier initiation is not yet described.

Objective: We assessed the early neurodevelopment of HIV+ infants who started ART within 21 days of life.

Method: Participants were enrolled from the public sector birth HIV-diagnosis programme. Inclusion criteria included the following: birth weight >2000 g, infant commencing ART <6 weeks and no infant cytomegalovirus disease. Antiretroviral therapy included Zidovudine/Lamivudine/Nevirapine for the first 2 weeks, the latter then replaced by Lopinavir/Ritonavir. Once body weight >3 kg and gestational age >44 weeks, Abacavir replaced Zidovudine. The Griffiths Mental Development Scales (GMDS) were administered at 10–12 months.

Results: Of 29 infants assessed, 23 (79%) were girls. Mean birth weight was 3002 ± 501g. Twenty-four mothers (83%) received ART during pregnancy. Seven (24%) infants were diagnosed HIV+ within 48 h of birth. Median [interquartile range] viral load (VL) at diagnosis was 3904 [259–16 922] copies/mL, age starting ART was 6.0 [3–10] days and age at VL suppression was 19.1 [15–36] weeks. At the GMDS assessment, nine (31%) participants had detectable VL and 26 (90%) had WHO clinical stage I disease. Griffiths Mental Development Scale was performed at a mean age of 11.5±0.8 months. Mean quotients were within the average range: Global Griffiths score was 103.6±10.9 and mean quotients on the subscales ranged from lowest 95.9±13.4 for locomotor to highest 112.8±11.3 for hearing-and-language.

Conclusion: Preliminary findings in this small group suggest that early neurodevelopmental scores are within the normal range in infants with perinatal HIV infection who started ART at a median of 6 days.

Introduction

HIV-infected (HIV+) children are at risk for neurodevelopmental delay. Neurologic insults develop after HIV enters the brain, creating an inflammatory state affecting neuronal and astrocyte growth and development[1]; the most severe manifestation being HIV encephalopathy (HIVE). HIV encephalopathy rates range from 6% to 30%, with higher rates in low- and middle-income countries especially with delayed initiation of combination antiretroviral therapy (ART).[2,3,4,5] These consequences place a great burden on the social and healthcare systems.

Antiretroviral therapy has decreased the incidence of HIVE,[6] and when initiated between 3 and 9 months of age, it improves clinical and neurodevelopmental outcomes.[7,8,9,10] Nevertheless, studies describing permanent deficits or lack of catch-up suggest that prevention is better than reversal of HIVE.[11,12,13] From 2016, the World Health Organization (WHO) introduced birth testing and recommended starting ART as soon as possible once HIV infection is confirmed.[14] Since the report of temporary remission in the Mississippi baby after very early ART,[15] there is increasing evidence that early ART in perinatally infected children improves infant outcomes.[16] Early ART can limit HIV reservoir size, and when started before 2 months of age, it is associated with fewer infected and transcriptionally active cells and less infectious virus recovery.[17,18,19,20,21,22,23,24] However, administering ART in a neonate and young infant is not easy with potential drug resistance because of under-dosing, or neurotoxicity because of overdosing.[25] The long-term outcomes of very early exposure to ART are still unknown. It is therefore imperative that neurodevelopmental testing be undertaken after early ART initiation. Our aim was to determine the neurodevelopmental outcomes of perinatally HIV-infected children after initiating ART within the first 3 weeks of life.

Research methods and design

We report early data from a prospective descriptive study conducted in the Family Centre for Research with Ubuntu (FAM-CRU) in Tygerberg Hospital, Cape Town South Africa, with recruitment from the Médecins Sans Frontières service in Khayelitsha and elsewhere in the public sector. Antiretroviral therapy was started as soon as HIV

infection was confirmed. HIV diagnosis was made by quantitative HIV-1 viral load (VL) testing and confirmed by a qualitative HIV-1 RNA PCR. Indeterminate samples were repeated until HIV diagnosis confirmation.[26,27] Inclusion criteria were the following: birth weight >2000 g, commencing ART <6 weeks of age and no infant cytomegalovirus (CMV) infection. Mothers or legal guardians were consented in person in their language of choice according to Good Clinical Practice standards.

Participants were seen as frequently as needed until stable, monthly for 3 months and then 3 monthly. Visits included a medical examination, growth monitoring, adverse event assessment and social work support where needed. At each visit, a pharmacist calculated the percentage adherence for each drug from returned ART containers and an adherence counsellor established reasons for over or under-dosing with the parent or caregiver, offered advice on problems identified and reviewed measuring techniques. HIV viral load was performed at baseline, 3, 6 and 12 months. Undetectable VLs were reported as <100 or <40 copies/mL depending on the blood volume available for testing. CD4 cell counts were done at 3, 6 and 12 months. Antiretroviral therapy comprised Zidovudine, Lamivudine and Nevirapine, with Lopinavir/Ritonavir replacing Nevirapine after 2 weeks of age or gestational age of 42 weeks. Once weight exceeded 3 kg and gestational age was above 44 weeks, Abacavir replaced Zidovudine. Participants also received co-trimoxazole from 6 weeks of age.

The Griffiths Mental Development Scales (GMDS) (0–2 years) were conducted by the same developmental paediatrician (BL) at 10–12 months of age.[28] The GMDS assesses five subscales: locomotor, personal–social, hearing-and-language, eye–hand co-ordination and performance (visual–motor abilities). A global score, the General Griffiths, is also calculated. Raw scores are converted into quotients, derived from norms of healthy British children, with a mean of 100 and standard deviation (SD) 16. While the GMDS is neither standardised nor validated in South Africa, it is the most widely used developmental assessment tool, is considered culturally fair and is used to assess young children including those exposed to HIV.[29,30,31,32,33,34,35,36] Vision was assessed clinically during testing and through the ability to track small cake decorations ('hundreds and thousands' test), which implies visual acuity of 6/24 or better.[37]

Statistical analysis was performed using Stata release 11 (StataCorp, College Station, TX) and Statistica 13 (software.dell.com. Dell Inc. 2015). For descriptive statistics, mean and SD were reported for normally distributed data and median and interquartile range (IQR) for skewed data. Guided by distribution of the data, Spearman and Pearson correlations were used to explore correlation between various parameters and neurodevelopmental outcomes. For calculating age at VL suppression, those who had not yet achieved VL suppression were assigned a date 2 days after the GMDS. Regression analysis explored the contribution of five predictors of GMDS scores: birth weight, ART start age, baseline VL, baseline CD4% and age at first VL suppression.

Descriptive data and GMDS scores were also compared to those from the early treatment arms on Children with HIV Early antiRetroviral (CHER) participating in a neurodevelopmental sub-study who received early ART from a median of age of 7.7 weeks and were assessed by the same investigators at 11 months of age.[10]

Results

Of 29 children studied, 23 (79%) were female. Mean birth weight was 3002 ± 501 g and gestation was 37.9 ± 2.3 weeks. HIV+ diagnosis was made by 48 h of birth in 7 (24%) and within 7 days of birth in 17 (59%) infants. Median [IQR] age for starting ART was 6.0 [3–10] days (range 0–21) from birth. Twenty-three achieved VL suppression at median [IQR] 19.1 [14.7–35.9] weeks of age (range 2–53) (Table 1).

Griffiths Mental Development Scale was performed at a mean of 11.5 ± 0.8 months (range 10.2–13.1) and scores are described in Table 2. Mean GMDS quotients were in the average range and within 1 SD of the standardised scores. The locomotor subscale had the lowest mean quotient. No children were suspected of having hearing or vision problems.

Clinical status at the time of GMDS is described in Table 3. One child had progressed to WHO stage II HIV disease (persistent oral candida), and two to stage III (chronic suppurative otitis media and pulmonary TB). Nine children (31%) had detectable VL at the time of GMDS testing, six (21%) had not yet achieved viral suppression and three had previously suppressed (one at 27 weeks and two at 19 weeks of age), but

rebounded to log 5.44 (273 328 copies/mL), log 3.18 (1519 copies/mL) and log 4.46 (28 649 copies/mL), respectively. Another participant suppressed at 3 months, and had a viral blip to 118 copies/mL at 6 months, with the VL undetectable 6 months later at GMDS.

A number of demographic and exposure issues with potential to influence neurodevelopmental outcomes were identified. These included two without antenatal care, one with an unsupervised home birth and three with maternal substance abuse: two methamphetamine and one methamphetamine and alcohol (over time these children were fostered by caring relatives). Medical problems included one each of the following: congenital pneumonia of unknown aetiology, intrauterine growth retardation, neonatal jaundice above exchange transfusion levels (resolved without exchange), congenital syphilis with mild hypoxia and suspected seizure, mild birth asphyxia (low birth Apgar scores and cord blood pH=7.17) and suspected hypoglycaemia (but glucose level not recorded) (these data not shown in any table).

The following adverse events, which could negatively impact neurodevelopment, were documented before the GMDS assessment: six with otitis media (one had two episodes), six with anaemia and three with neutropenia (Zidovudine was discontinued). One infant recovered fully after treatment for suspected bacterial meningitis and another was hospitalised for 6 months with pulmonary tuberculosis. Lastly, failure to thrive because of poor feeding and insufficient caloric intake occurred in one infant.

Adherence was calculated at a median [IQR] of 10 [9–11] visits. Only one participant had acceptable adherence percentages for all drugs at all visits. Three participants had poor adherence for more than half of the visits, with the rest over or under-dosing at various times. For the former, the infant would spit syrups out and caregivers were unsure how much to replace. For the latter, the caregivers either measured syrups incorrectly or were non-compliant. This prompted clinicians to encourage treatment supporters for the caregivers.

TABLE 1: Demographic characteristics of participants (*N* = 29)**.**	
Sex	Female = 23 (79%)
Birth weight (g) Mean ± SD	3002 ± 501 (2150–4070)
Gestational age (weeks) Mean ± SD (range) (3 unknown gestation)	37.9 ± 2.3 (33–41)
Birth method: vertex delivery Caesarean section	21 (72%) 8
Mother's age at birth (years) Mean ± SD (range)	29.3 ± 5.4 (18.9–40.4)
History of prenatal substance exposure	2 – Methamphetamine 1 – Alcohol + methamphetamine
Home language	21 (70%) Xhosa 6 (21%) Afrikaans 1 Shona 1 English
PMTCT – mother	24 (83%) Yes 4 (14%) No 1 Unknown
Infant age HIV diagnosis (days) Median [IQR] (range)	6 [3–12] (0–52)
Infant age HIV diagnosis: within 48 h within 1 week	 7 (24%) 17 (59%)
Infant ART start age (days) Median [IQR] (range)	6.0 [3–10] (0–21)
ART regimen started *n* (%)	16 (55%) Zidovudine, Lamivudine, Nevirapine 6 (20%) Zidovudine, Lamivudine, Lopinavir/Ritonavir 7 (24%) Abacavir, Lamivudine, Lopinavir/Ritonavir
Infant baseline VL (copies/mL) Median [IQR] (*n*=26[a])	3904 [265–16 922] (range 99–201 916)
CD4 closest to baseline Median [IQR] Age (days) Absolute count CD4%	 14 [9–28] (range 0–251[b]) 1938 [1446–2570] (range 679–3776) 43 [35–56] (range 19.6–71)
Time to undetectable VL[c] (weeks from birth) Median [IQR] (range)	19.1 [15–34] (2–53) (*n* = 23[d])
[a]3 only had HIV PCR+ and no VL measured. [b]Participant only enrolled onto study at this age – only had VL before and no CD4 counts. [c]VL done at baseline/diagnosis, 3, 6, 12 and 18 months. [d]Six did not suppress by time of GMDS assessment.	

Source:Authors' own data compilation from this study

VL, viral load; IQR, interquartile range; ART, Antiretroviral therapy; PMTCT, prevention of mother-to-child transmission; SD, standard deviation; GMDS, Griffiths Mental Development Scale.

TABLE 2: Scores on Griffiths mental development scales (quotients) at mean age of 11.5 months (*n* = 29)**.**				
Scale	Mean	SD	Maximum	Minimum
Locomotor	95.9	13.4	125	74
Personal–social	104.2	14.7	138	72
Speech and hearing	112.8	11.3	131	85
Eye–hand coordination	105.0	17.5	136	60
Performance (visual–spatial)	99.1	15.7	133	68
General Griffiths	103.6	10.9	123	82
Norms from healthy British children: mean 100 ± 16				

Source: Authors' own data compilation from this study

TABLE 3: Clinical status at the time of neurodevelopmental assessments (*N* = 29).	
Age: Mean ± SD (range)	11.5 ± 0.8 months (range 10.2–13.1)
HIV disease severity: WHO categories	1 = 26 (90%) 2 = 1 3 = 2
Growth: WHO *z*-scores for age (mean ±SD) Weight Length Head circumference	 -0.09 ± 0.9 (range -1.7 to 1.6) -1.1 ± 1 (range -3.5 to 0.2) 0.23 ± 1 (range -1.9 to 2.4)
ART regimen	29 Abacavir, Lamivudine, Lopinavir/Ritonavir
VL undetectable, *n* (%)	20 (69%)
CD4 closest to GMDS Median [IQR] Absolute count CD4%	 2097.9 [743–1512] (range 863–3790) 33.8 [27–41] (range 18–53)
CD8 closest to GMDS Median [IQR] Absolute count CD8%	 1489 [1131–2437] (range 608–7551) 27 [21–34] (range 13–53)
CD4/CD8 ratio closest to GMDS Median [IQR]	1.33 [0.83–1.92] (0.38–3.79)
Current caregiver *n* (%)	23 (79%) mother 1 shared mother and grandmother 2 aunt 1 foster mother 2 grandmother
Father or father-figure present, *n* (%) Caregiver/father/father-figure Drugs or alcohol abuse, *n* (%) Housing, *n* (%): Brick Informal dwelling Electricity in house, *n* (%) Household receives social grants, *n* (%)	20 (69%) 7 (24%) 10 (34%) 19 (66%) 28 (97%) 26 (90%)

Source: Authors' own data compilation from this study

IQR, interquartile range; SD, standard deviation; GMDS, Griffiths Mental Development Scale.

Correlations between GMDS scores and possible predictors of developmental outcomes (birth weight, gestation, maternal age, baseline VL, age starting ART, time to suppression and CD4 parameters at baseline) were not significant. The five predictors of GMDS scores entered into the regression model also did not show significant relationships, that is, birth weight, ART start age, baseline VL, baseline CD4% and age at first suppression. CD8 count at the time of GMDS showed a negative correlation with personal–social (Pearson r=-0.41; p=0.03) and a negative trend with General Griffiths (Pearson r = -0.6; p = 0.06).

For growth parameters closest to the Griffiths assessment, head circumference z-scores correlated significantly with the Performance (visual–spatial) scores (Pearson's r = 0.4; p = 0.02) and weight z-score correlated with eye–hand coordination scores (Pearson's r = 0.36; p = 0.05). There was a positive trend between weight for age z-score and General Griffiths score (Pearson's r = 0.34; p = 0.07).

We compared the GMDS scores of those whose mothers had ART for prevention of mother-to-child transmission of HIV (PMTCT) and those who did not and found no difference between the groups (see Supplementary Table 1). There were also no significant differences on the GMDS scores between those with detectable VL and undetectable VL at the time of the test, despite the mean scores in the hearing-and-language and eye–hand coordination subtests being 5 points lower for the nine with detectable VL compared to the 20 with undetectable VL at testing (Table 4). We also compared the following participant demographics between the detectable VL and undetectable VL groups: birth weight, baseline VL copies and CD4 parameters, ART start age, CD4, CD8 and growth parameters at the time of GMDS, and found no difference (see Supplementary Table 2).

TABLE 4: Comparison of Griffiths mental development scale quotients in those with and without virological suppression at testing.

Viral load at testing	Detectable VL *n* = 9	Undetectable VL *n* = 20	*p**
Mean age at testing (months)	11.4	11.5	
Locomotor	96.9 ± 13.5	95.4 ± 13.7	0.65
Personal–social	102.2 ± 13.9	105.1 ± 15.2	0.48
Hearing-and-language	109.2.4 ± 10.2	114.4 ± 11.6	0.32
Eye–hand coordination	101 ± 18.4	106.9 ± 17.3	0.46
Performance (visual–spatial)	97.9 ± 14.9	99.1 ± 16.4	0.94
General Griffiths	101.8 ± 10.4	104.4 ± 11.3	0.52

Source: Authors' own data compilation from this study

VL, viral load.

* Mann–Whitney *U*.

Griffiths Mental Development Scales scores achieved by this cohort were similar to those from the CHER cohort (children on ART commenced at 7 weeks of age) at a mean age of 11.3 months, apart from personal–social subscale, where the CHER cohort had mean quotients 7 points above that of the current study population (Table 5). Post hoc item comparison for personal–social showed that CHER participants were more likely to help with dressing, hold an open cup for drinking, try to use a spoon for feeding and obey simple requests. Participants on the current study were more likely to clap hands and enjoy an adult showing a book.

Significant differences between the two groups are shown in Table 5, with CHER having higher VLs and lower CD4 counts at baseline and a longer time to undetectable VL compared to participants in the current study. Abacavir also replaces Zidovudine use in the CHER participants.

TABLE 5: Comparison between study participants and Children with HIV Early antiRetroviral early treatment participants.[1]			
	Current study	**CHER early ART**	***p***
Number enrolled	29	64	
Age of ART initiation Median [IQR]	6.0 [3–10] days	7.7 [7.1–9.5] weeks	<0.001
Birth weight (g)	3002 ± 501	2994 ± 406	0.98
Gestational age (weeks)	37.9 ± 2.3 (3 unknown)	38.9 ± 2.3 (3 unknown)	0.06
PMTCT – mother Yes No Unknown	 24 (83%) 4 (14%) 1 (3%)	 55 (86%) 6 (9%) 3 (5%)	
History of prenatal substance exposure	2 Methamphetamine 1 Alcohol+methamphetamine	2 Alcohol	
Infant baseline VL (copies/mL) CD4 absolute count CD4%	 2494 ± 47629 (*n* = 26) 2090 ± 800 44.7 ± 14.2	 5 500 942 ± 55 693 2062 ± 1100 35.2 ± 8.6	 <0.01[a] 0.42 <0.01
Time to undetectable VL[b] (weeks)	30.0 ± 16.6 (*n* = 23)	38.8 ± 8.8	0.01
ART regimen at the time of test	28 Abacavir, Lamivudine, Lopinavir/Ritonavir 1 Abacavir, Lamivudine, Didanosine	63 Lamivudine, Lopinavir/Ritonavir, Zidovudine 1 Abacavir, Nevirapine, Didanosine	
VL undetectable at GMDS test	20 (69%)	40 (62%)	
Age at GMDS (months)	11.5 ± 0.8	11.3 ± 1.1	0.16
GMDS quotient scores:			
Locomotor	95.9 ± 13.4	97.7 ± 12.5	0.3
Personal–social	104.2 ± 14.7	111.2 ± 13.5	0.04
Speech and hearing	112.8 ± 11.3	112.5 ± 10.4	0.89
Eye–hand coordination	105.0 ± 17.5	107.4 ± 15.8	0.66
Performance (visual spatial)	99.1 ± 16.1	100.3 ± 13.1	0.4
General Griffiths	103.6 ± 11.0	106.2 ± 10.4	0.21
Norms from healthy British children: mean 100 ± 16			

Source: Ref [1]. Authors' own data compilation from this study combined with previous work (reference no 10) VL, viral load; IQR, interquartile range; ART, Antiretroviral therapy; PMTCT, prevention of mother-to-child transmission; SD, standard deviation; GMDS, Griffiths Mental Development Scale; CHER, Children with HIV Early antiRetroviral.
Results expressed as mean ± SD. [a] Mann–Whitney *U*. [b] For those not yet suppressed at assessment, date for suppression was allocated 2 days after assessment date.

Discussion

These findings from the first 29 infants who started ART at a median age of 6 days are encouraging and show potential for normal neurodevelopmental outcomes, despite other medical conditions in nine infants that may impair neurological development. These scores are well within 1 SD of the UK norms, and are comparable to other South African infants assessed at similar ages using the GMDS[29,30,32,33,34] (see Supplementary Table 3 for summary of scores). This finding is despite almost a third not being virologically suppressed at testing. However, VLs in this cohort indicated low exposure to HIV because of maternal ART.[38]

We previously described neurodevelopmental outcomes in the CHER trial at 11 months.[10] We compared children on delayed ART to those who started early ART at a median [IQR] of 7.7 [7.1–9.5] weeks. The GMDS scores from the CHER early treatment arms are comparable to this very early treatment group, apart from the personal–social subscale (Table 5), which is the most subjective as caregiver report items are used, and may reflect a change in child-rearing practices over time with less emphasis on self-care skills. The CHER early treatment arms had a mean baseline VL of $\log_{10}$ copies/mL 5.64 which is far higher than the current study and baseline mean CD percentage of 35% which is lower than the current study, and longer time to undetectable VL. This may suggest that there is a safe window period for starting ART – between birth and a median of 7.7 weeks; however, these are early neurodevelopmental outcomes. Alternatively, were it not for adverse *in utero* exposures and non-suppressed VLs in six infants, the scores may have been higher than CHER early treatment participants. The early diagnosis of HIV+ infants within 48 h in 24% and by 7 days of age in 59% reflects high proportion of prenatal HIV –infection, which also negatively impacts outcomes. In the CHER trial, *in utero* infection could not be assessed as infant screening began at 4–6 weeks of age for HIV.

An important finding is that we identified a number of challenges within the context of perinatal HIV infection, despite good PMTCT programmes. In those perinatally infected infants, a number of secondary effects, including systemic illnesses and environmental affects, may negatively impact a child's early neurodevelopment.[39,40,41] In our sample,

we identified three with no prenatal care, three substance abuse, two congenital infections (syphilis and pneumonia of unknown aetiology), one co-infected with tuberculosis and one nutritional failure. Growth in participants was appropriate for weight and head circumference, but mean length *z*-score was -1.1.

We noted variability of ART adherence and the delay in attaining competence with ART dosing and adherence, with six children not yet suppressed at the time of GMDS assessment. Management of these young children was challenging as caregivers were non-compliant, under-skilled and found difficulty administering liquid formulations. Solid or dispersible formulations would certainly improve adherence.[42,43] Our findings do not suggest neurotoxicity from ART.

This work had some limitations. As multiple factors may influence outcomes, 29 children starting ART very early are too few to assess weak associations with neurodevelopmental outcomes, including our finding of lower locomotor scores compared to other subscales. More girls than boys were enrolled in the sample; although previously described,[44] this may be because of small sample size. We were not able to determine reliable predictors for neurodevelopmental outcomes, or compare the outcomes of suppressed and unsuppressed participants. This was also hindered by time to suppression being inaccurate as VLs were only done at baseline 3, 6 and 12 months. We did not collect information on maternal health, immune status, VL or antiretroviral therapies. In the absence of South African normative data on the GMDS, a control or comparison group would have been helpful. However, we have experience in this community using the GMDS and are able to use these for comparison[30,32] (Supplementary Table 3). The confounding problems of mothers with substance abuse did not seem to have a major impact, but the limitation is probably sample size.

Our findings are relevant to upscaling neonatal HIV identification and care.[45,46] While the number of HIV+ infants is decreasing, this population remains at high risk because of structural and behavioural challenges in providing appropriate care. As liquid Lopinavir/Ritonavir formulation is poorly tolerated, newer formulations and other alternatives such as integrase inhibitors will be better accepted. Healthcare planners should not downscale programmes according to decreasing numbers, as those failing

PMTCT require a higher level of care and intensive intervention to enable benefit from early ART. With the potential of early ART to limit HIV reservoir seeding, and potential to contribute to functional cures, treatment programmes need to support these vulnerable infants and their caregivers.[47] Mentor mothers as treatment supporters may decrease the burden of HIV care and consequences of developmental delay, and could be very important when planning programmes. If these needs can be met, our findings are encouraging.[48,49]

Conclusion

Preliminary findings in this small group suggest that despite PMTCT failure, children infected perinatally with HIV may have typical neurodevelopment if starting ART at a median age of 6 days, and similar to those starting ART at a median of 7 weeks. Good supportive care, including for ART adherence, is essential. A larger cohort that includes controls is in study and the findings at 18 months of age will inform on the influence of time to VL suppression and reservoir size and also the influence of social factors and demographic factors on neurodevelopmental outcomes. This may also allow for more precise study of locomotor outcomes.

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Supplemenatry materials:

SUPPLEMENTARY TABLE 1: Comparison of Griffiths mental development scales quotients in those whose mothers had antiretroviral therapy for prevention of mother-to-child transmission of HIV or not reported as mean (standard deviation).

	Yes PMTCT *N* = 24	No PMTCT *N* = 4	*p*
Locomotor	96.9 (14)	92 (15)	0.51
Personal–social	104.0 (15)	111.8 (6)	0.22
Hearing-and-language	112.1 (12)	118.0 (8)	0.34
Eye–hand coordination	107.5 (16)	101.5 (7)	0.38
Performance (visual–spatial)	101.5 (15)	90 (4.7)	0.06
General Griffiths	104.6 (11)	103.3 (3)	0.84

Source: Authors' own data compilation from this study
PMTCT, prevention of mother-to-child transmission.

SUPPLEMENTARY TABLE 2: Comparison of participant characteristics for those who had detectable and undetectable viral loads at Griffiths mental development scales testing reported as mean (standard deviation).

Viral load at testing	Detectable VL *n* = 9	Undetectable VL *n*=20	*p*
Birth weight	2912.2 (576)	3042.5 (475)	0.37
Baseline VL copies	45105 (70493) (*n* = 8)	16000 (318345) (*n* = 18)	0.36
ART start days from birth	9.3 (6.4)	5.8 (3.8)	0.14
CD4 closest to GMDS Absolute count CD4%	 2061.2 (1035) 30.8 (2.7)	 2180.8 (560) 35.4 (8.1)	 0.25 0.21
CD8 closest to GMDS Absolute count CD8%	 2348.1 (2092) 30.2 (8.7)	 1723.7 (948) 26.3 (10.5)	 0.56 0.26
CD4/CD8 ratio closest to GMDS	1.2 (0.6)	1.6 (0.8)	0.21
CD4%/CD8% ratio closest to GMDS	1.15 (0.6)	1.6 (0.9)	0.17
CD4 baseline Absolute count CD4%	 2040.6 (816) 40.0 (12.4)	 2099.6 (818) 47.1 (14.4)	 0.69 0.26
Growth: WHO *z*-scores for age Weight Head circumference Length	 -0.0 (1.1) 0.37 (0.8) -1.02 (1.1)	 -0.13 (0.8) 0.17 (1.1) -1.13 (1.0)	 0.87 0.52 0.71

Source: Authors' own data compilation from this study
ART, Antiretroviral therapy; GMDS, Griffiths Mental Development Scale; VL, viral load.

SUPPLEMENTARY TABLE 3: Summary of scores on the Griffiths mental development scales locomotor and General Griffiths from controls on South African studies at similar ages.			
Author/Study	Age	Locomotor	General Griffiths
Perez: non-anaemic controls[34]	9 months	136	127
Davies: non-foetal alcohol syndrome controls[33]	7–12 months	100	104
Laughton: HIV exposed uninfected controls[10]	11 months	102	107
Current study	**11 months**	**95.9**	**103.6**
Amod: South African sample[29]	13–16 months	98	102
Springer: HIV exposed uninfected controls[32]	17–19 months	87	87

Conclusion and future directions:

The aim of this dissertation was to explore the effects of different ART treatment strategies on the early neurodevelopment of children perinatally infected with HIV. We compared the neurodevelopment of participants on various ART treatment strategies on a prospective longitudinal study and on a cross-sectional pilot study and found that:

- Early ART is better than deferred ART for early motor and global development
- Commencing ART early (before 12 weeks of age) followed by closely planned and monitored ART interruption at 1 year or 2 years of age, using CD4 counts, appeared safe as neurodevelopmental outcome at 5 years was similar to uninfected neighbourhood controls.
- Perinatally infected children, starting ART in the first few days of life, have normal developmental outcomes at 11 months of age.
- Early developmental delay may recover after starting ART.
- HIVE may develop regardless of ART treatment strategy, which may resolve, and this requires further investigation.
- Visual perceptual deficits at 5 years of age may develop regardless of ART treatment strategy

These findings have contributed to contemporary knowledge of ART treatment strategies for children perinatally infected with HIV.

There was no evidence to support toxicity of ART to the developing brain, or possibly the benefit may outweigh the risk, with minor effects undetected on the GMDS. We should continue to monitor for effects of ART.

Our findings are supported by more recent studies showing improved neurodevelopment in infants treated early and with early viral suppression, suggesting that starting ART at 4 – 6 months of age may be too late.[53-57] Further studies have also support planned treatment interruption in older children.[58, 59]

In five-year old children perinatally HIV infected, visual perceptual deficits were recognised regardless of ART treatment strategy. This finding is concerning as visual perceptual abilities are important for future educational outcomes.[60] Further

investigation is required to determine the timing of pathogenic pathways to this neurologic insult in order to identify predictors of poor outcome to prevent or remediate as early as possible.

The small sample sizes in the comparison groups for neurodevelopmental outcome, as well as a survivor effect in the delayed treatment group, are a limitation to interpretation of results. Important co-morbid factors such as poverty, nutrition and child and maternal illnesses, which may have impacted on neurodevelopment were only addressed superficially, although were mitigated by randomization of arms in the CHER trial.

While we may infer that many children were prenatally infected with HIV, we did not have documented evidence in most cases, and some infants may have been infected during birth or afterwards through breast feeding.

This study did not address the effects of different antiretroviral drugs and CNS penetration, as most children were on the same regimen.[43, 61] Other HIV related factors should also be considered in future studies such as: viral and host genetics, time to viral suppression, reservoir size formation and the host immune response. Longer term outcomes are needed to address effects on brain maturation and children's abilities that cannot be measured in the pre-school age group.

It is essential to note that there are multiple influences on a child's neurodevelopment of which timing of ART initiation is only one.[62] Contextual factors such as prenatal care of mothers, maternal nutrition and mental health are important. For the child, a nurturing home environment, adequate nutrition and timeous intervention for illnesses and developmental delay all play a role in early neurodevelopmental outcomes.[63-65]

Considering the above findings, future studies should be conducted to assess the longer-term effects of different early ART treatment strategies on childhood cognitive, behavioural and educational outcomes. Early effects on the structural foundations of the developing brain may only become apparent when measuring skills that are expected to develop in an older child. These deficits are described in HIV infected school-aged African children who started ART after 6 months of age.[66] Further studies should include measuring the effects on neurodevelopment of the viral reservoir, host inflammatory response and the influence of contextual factors as mentioned above. Identifying early

predictors of poor outcomes are the first step for planning effective intervention programs.

In conclusion, starting children on ART whilst asymptomatic has encouraging neurodevelopmental outcome at 5 years, apart from visual perceptual deficits which are noted regardless of ART treatment strategy. Planned treatment interruption does not affect outcome by 5 years of age, however this needs careful clinical guidance. Our findings support global initiatives for decreasing the HIV testing and treatment gaps for infants and children.[40, 67] Longer term outcomes in older children will continue to inform on ART treatment strategies.

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Appendices:

Appendix 1:

Table 3. Nomenclature and research case definitions for neurologic manifestations of human immunodeficiency virus-type 1 (HIV-1) infection. (from: Janssen RS, Cornblath DR, Epstein LG, Foa RP, McArthur JC, Price RC and the working group of the American Academy of Neurology AIDS Task Force. *Neurology* 1991,41:778-785.)

HIV-1-Associated progressive encephalopathy of childhood

Probable (must have each of the following):

1) Evidence for systemic HIV-1 infection:
   a) Infants and children <15 months
      i) Virus in blood or tissues, or
      ii) Presence of HIV-1 antibody
      and
      evidence of cellular and humoral immune deficiency
      or
      other conditions meeting CDC case definition for AIDS
   b) Children ≥15 months
      i) Antibody or virus in blood or tissues
2) At least one of the following progressive findings present, at least 2 months
   a) Failure to attain or loss of developmental milestones or loss of intellectual ability, verified by standard developmental scale or neuropsychological tests.
   b) Impaired brain growth (acquired microcephaly or brain atrophy demonstrated on serial CT or MRI)
   c) Acquired symmetric motor deficits manifested by two or more of the following: paresis, abnormal tone, pathological reflexes, ataxia, or gait disturbance
3) Evidence of another etiology, including active CNS opportunistic infection or malignancy, must be sought from history, physical examination, and appropriate laboratory and radiologic investigation (dg. Lumbar puncture, neuroimaging). If another potential etiology is present, it is *not* thought to be the cause of the above cognitive/motor/behavioural/developmental symptoms and signs.

Possible (must have *one* of the following):

1) Other potential etiology present (must have *each* of the following):
   a) As above (see *Probable*) #1 and 2.
   b) Other potential etiology is present but the cause of #2 is uncertain
2) Incomplete clinical evaluation (must have *each* of the following):
   a) As above (see *Probable*) #1 and 2.
   b) Etiology cannot be determined (appropriate laboratory or radiologic investigations not performed).

Appendix 2:

Sample size calculation:

The locomotor subscale was used as it was expected that locomotor development would have the worse outcome. Griffiths scores are standardised with a mean of 100 and standard deviation of 15.

Table 1: Predicting expected mean quotients (locomotor subscale) using results from other studies

Age at Griffiths testing	**10 – 11 months**	**24 – 26 months**	**32 months**	**60 months**
Arm 1 (delayed therapy)	80[1]	80[1]	67[4]	68[5]
Arms 2 & 3 combined (hoping there will be no difference)[6]	90	92	92	87
Exposed and uninfected controls [2]	100	100	98	96
Unexposed and uninfected controls[3]	110	110	110	110

Notes for Table 1:

1. Results from children on different Nucleoside Antiretroviral Regimes, (these are results of the Bayley on children < 30.5 months of age). The baseline before starting treatment was 80 ± 21 ,(Raskino et al 1999)

2. Molteno et al (1991) in their article on the Preschool development of children in Cape Town, showed that at 12 months development correlated with family stability, and at 30 months it was associated with mother's education and family stability. The Griffiths scores can also look good at 1 year and then deteriorate it is part of "natural decline".

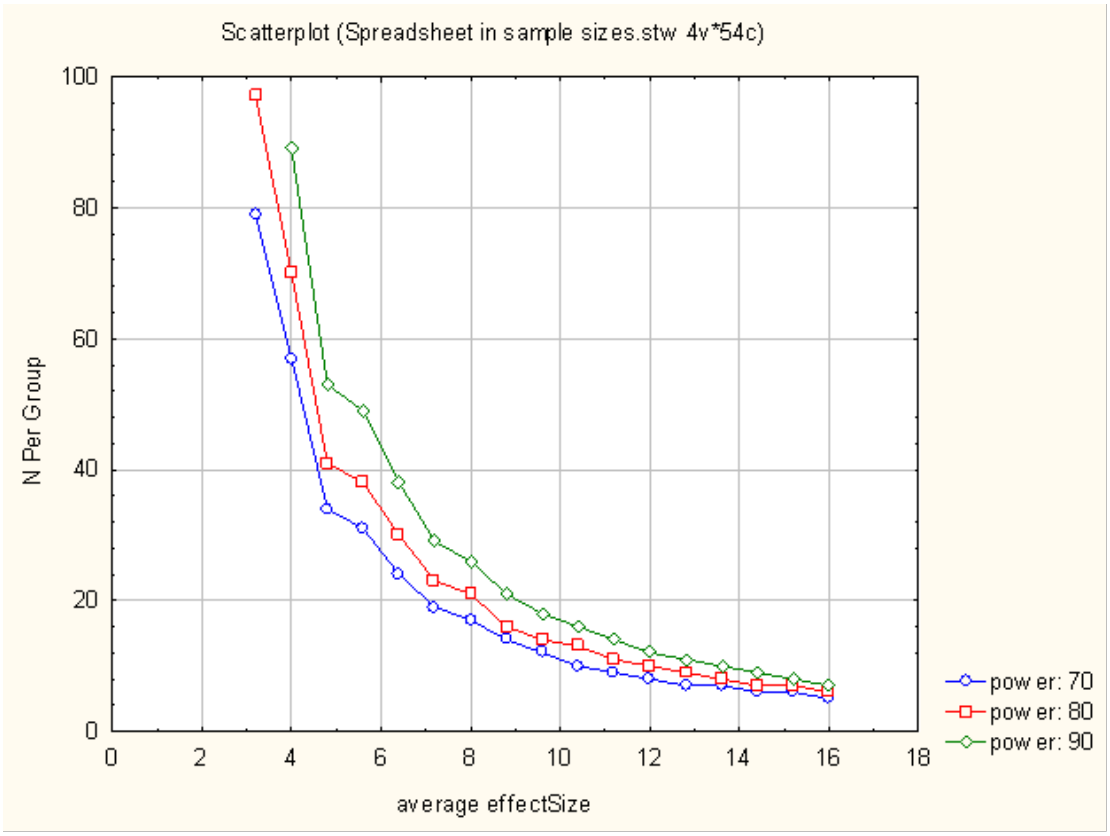
3. South African children tend to achieve their motor milestones earlier than average

4. Smith showed the mean baseline for Locomotor on the Griffiths was 67 for locomotor before starting HAART on children at Red Cross Hospital, Cape Town. At follow up after 6 months the scores had not improved. (19 of the 26 children assessed were below 4 years of age).

5. Smith also showed that the patients did not improve after treatment, although they remained static and did not deteriorate further (This is a bit of a skewed result as there were some who did worse who were not tested and not included in the results - those that died or were not too ill to be tested).

6. Shanbhag et al (2005) studied the effects of combined therapy on neurocognitive functioning. For those diagnosed as neurocognitively healthy, neurocognitive scores remained stable over time with a mean standard score of 89.6 ± 11.8 at first evaluation and 91.9 ± 11.9 at most recent evaluation. I am going to use these scores for the children on early treatment, i.e. they seem neurocognitively healthy to us but there are deficits. Combined results were also given for the whole group (neurocognitively healthy and encephalopathies) born after January 1996 and their score was 87.2 ± 10.49, hopefully our patients will be well, and stable until 3.5 years and only at 5 years we will start picking up the problems.

Figure 1 shows the sample size vs average effect size for the different power values for 5 groups. For a sample size of 40 per group, we should be able to pick up an average effect size of 6.4 as significant with a power of 90%.



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Presentations at International conferences related to this research:

B Laughton. 12th International Scientific meeting of the Association for research in infant and child development. London, England. 14 May 2010. Invited Speaker: The neurodevelopmental outcome of HIV-infected infants on Antiretroviral Therapy.

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List of abbreviations:

ART	Antiretroviral therapy
ART – 40W	CHER study arm starting antiretroviral therapy early and interrupting at 40 weeks on study (close to first birthday)
ART- 96W	CHER study arm starting antiretroviral therapy early and interrupting at 96 weeks on study (close to second birthday)
ART- Def	CHER study arm starting antiretroviral therapy when clinical or immunological criteria are met (ART is deferred)
CDC	Centers for Disease Control and Prevention (CDC)
CHER	Children with HIV early antiretroviral trial
CHEU	Children perinatally HIV exposed and uninfected
CHEU	Children perinatally HIV unexposed and uninfected
CNS	Central Nervous System
CSF	Cerebrospinal fluid
GMDS	Griffiths Mental Development Scales
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HIV+	HIV-positive
HIVE	HIV encephalopathy
PMTCT	Prevention of mother to child transmission